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SOME TRANSFORMATIONS OF ANNOTININE

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Some Transformations of Annotinine", submitted by Peter William Braithwaite, B. Sc.(Hons.), in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Efforts to determine the structure of the *Lycopodium* alkaloid, lycopodine, by direct interrelationship with an alkaloid of known structure, annotinine, are described. Although a correlation was not obtained, several interesting reactions of annotinine were encountered and investigated; in particular, the reaction of annotinine with potassium methoxide. The structure of the "abnormal" product of this reaction was investigated in detail.

Methyl desoxidoannotinate and methyl *epi*desoxidoannotinate have been prepared and shown not to be identical with dihydroolivine, the reduction product of a new *Lycopodium* alkaloid.

ACKNOWLEDGEMENTS

The author extends his profoundest thanks;

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CONTENTS

DISCUSSION OF RESULTS

The alkaloid, lycopodine $C_{16}H_{25}ON$, was first isolated by Boedeker (I) in I88I from the club moss *Lycopodium complanatum* L. and since that time approximately 40 more alkaloids have been isolated from several species of the *Lycopodium* family.

At the time that this work was initiated, (October, I958), the structure of only one of the *Lycopodium* alkaloids was unequivocally known. Wiesner (2) and co-workers had shown in I957 that annotinine $C_{16}H_{21}O_3N$ had the structure I (chart I, page 6). Of the other alkaloids, lycopodine was by far the most accessible and consequently much more was known of its chemistry than of the minor alkaloids.

Manske and Marion (3) in I942, showed that selenium dehydrogenation of lycopodine yielded 5:7 dimethylquinoline and 7-methylquinoline. Under the conditions used for the selenium dehydrogenation, it was possible that ring fission, followed by ring formation had occurred but dehydrogenation experiments (3) under milder conditions made this theory seem unlikely, since the formation of 7-methylquinoline with palladium and barium sulphate and also with phthalic anhydride was observed. Hence lycopodine presumably contains a reduced 7-methylquinoline system.

MacLean and co-workers (4,5 and 6) in the I950's obtained ring cleavage of lycopodine by the von Braun method and

performed several series of experiments on the cleavage products, which showed that lycopodine possesses the hexahydrojulolidine ring system XI (see page 4), that is present in annotinine.

It was also inviting (in 1958) to think that since both lycopodine and annotinine contained 16 carbon atoms that their carbon skeletons were identical XII (see page 4).

In 1950, MacLean, Manske and Marion (7) had shown that the single oxygen function in lycopodine was ketonic and MacLean and co-workers (6) in 1956 showed that it was flanked by a methylene group. This could easily arise biogenetically through the oxidation of the potential hydroxyl group of the lactonic group of annotinine I (chart I, page 6).

The number of C-Me groups in lycopodine was not known in 1958 and so it was also inviting to propose that the carbon atom of the lactonic carbonyl group in annotinine was fully reduced to a methyl group in lycopodine. The epoxide of annotinine can not be present, since lycopodine contains only one oxygen atom and hence a plausible structure for lycopodine was structure VII (chart I, page 6). The stereochemistry of lycopodine was assumed to be identical to that of annotinine.

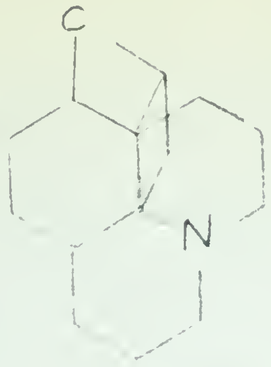
The fact that selenium dehydrogenation of the structure VII could not give 7-methylquinoline directly, does not necessarily invalidate structure VII, since 7-methylquinoline was also obtained by dehydrogenation of annotinine.

ATTEMPTED CONVERSION OF LYCOPODINE TO A 1:2 DIONE.

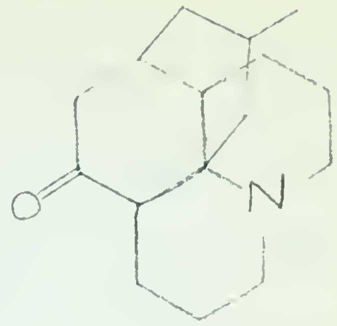
The source of lycopodine was Lycopodium clavatum L. from which a crude mixture of alkaloids was obtained, by using the proceedure of Manske and Marion (9). A solution of the crude alkaloids was chromatographed on basic alumina. Benzene elution yielded crude lycopodine from which pure lycopodine, I.R. ($CHCl_3$) 1701 $cm.^{-1}$ was obtained by recrystallisation from ether. The remaining lycopodine was purified as the perchlorate.

The frequency at which the keto group in lycopodine absorbs in the infrared is worthy of comment. It was originally reported (7) that lycopodine absorbs at 1693 $cm.^{-1}$. Since this value is outside the range 1705 - 1725 $cm.^{-1}$ normally associated with unstrained ketones, it was not possible to immediately assign the keto group to a six-membered ring. In fact, the frequency agrees better with that usually observed in a medium sized (i.e. 7 to 10 membered) ring. Our measurements showed that lycopodine absorbs at 1701 $cm.^{-1}$ in $CHCl_3$ solution and nujol mull, and 1705 $cm.^{-1}$ in CCl_4 solution. Since the 1705 - 1725 $cm.^{-1}$ region for unstrained ketones refers to measurements made in non-polar solvents such as carbon tetrachloride, the carbonyl frequency is not in disagreement with structure VII. It is interesting to note that lycopodine hydrobromide absorbs at 1718 $cm.^{-1}$. The difference in frequency between the salt and the free base suggested the possibility of an interaction between the unshared

XI



XII



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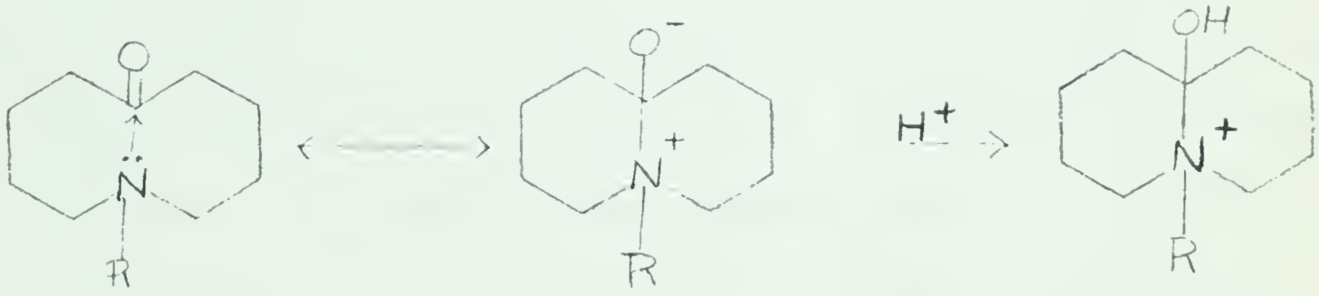
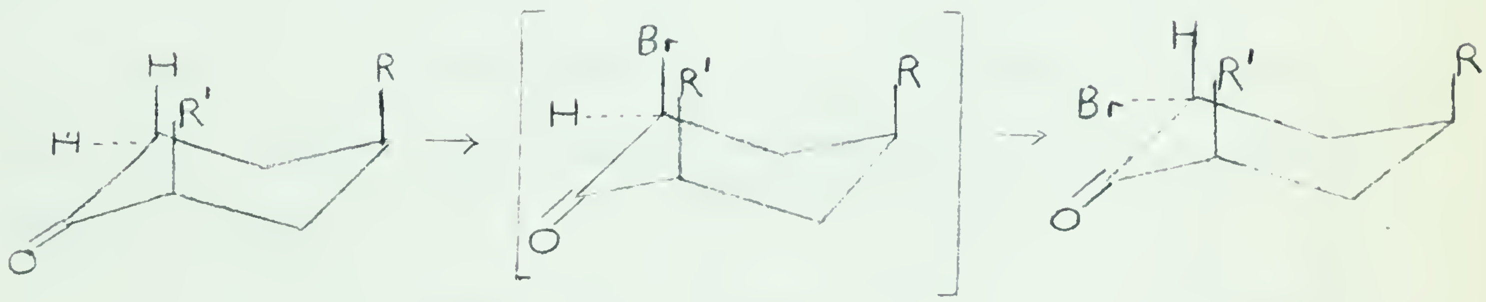
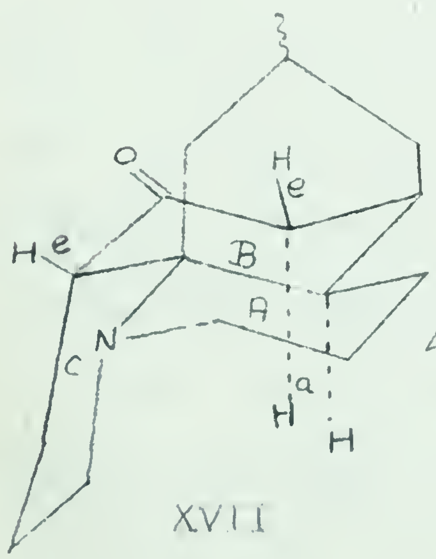
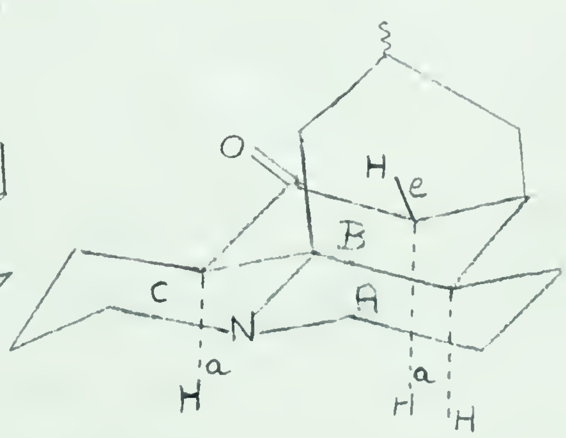


XIII XIV XV

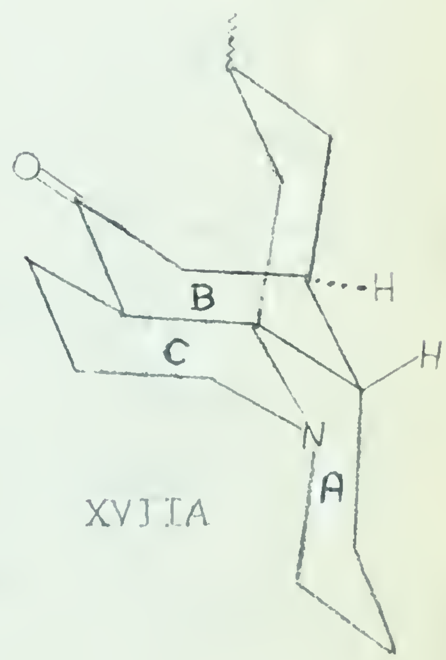




XVII



XVIII



XVIIA

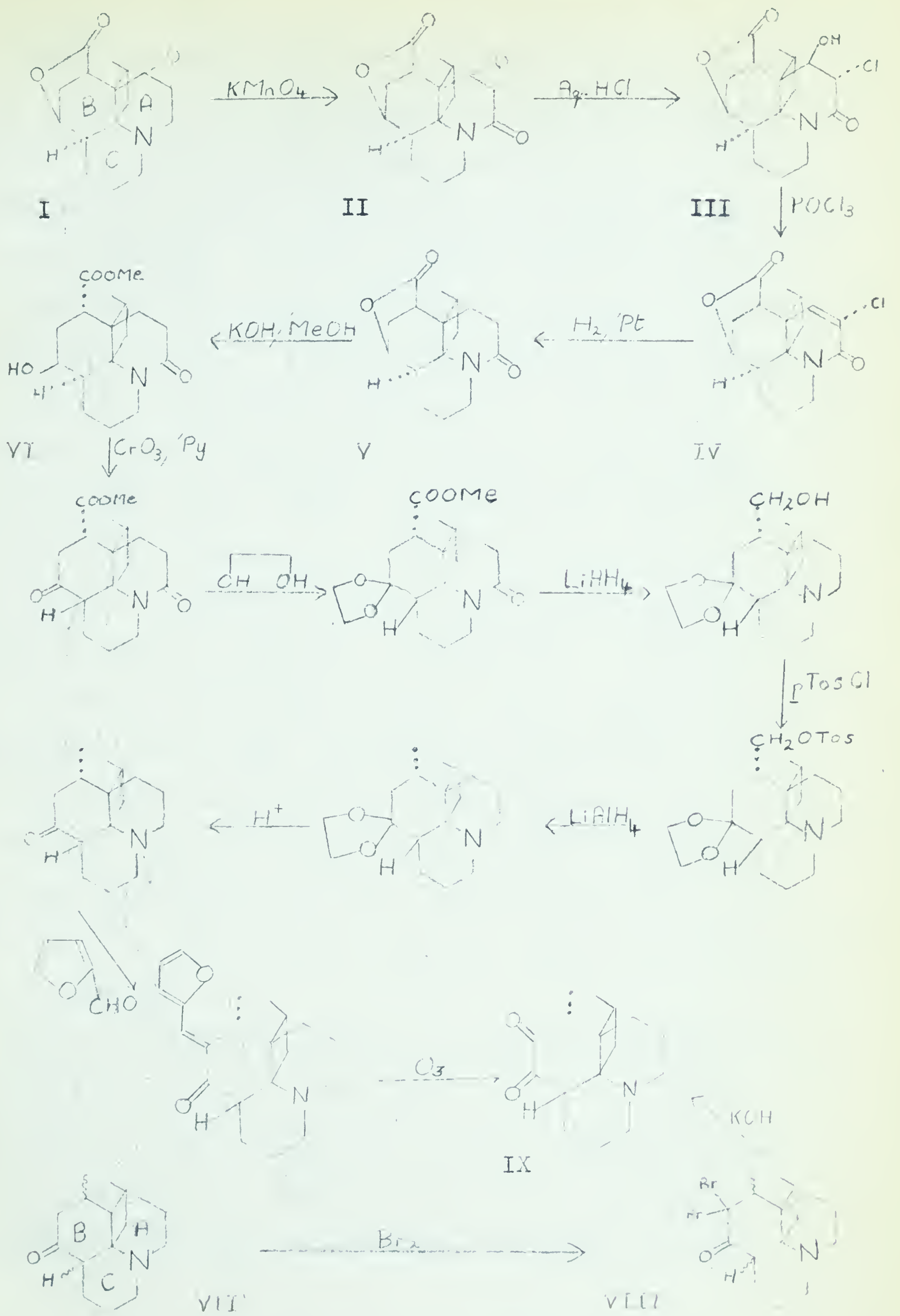
electrons on the nitrogen and the carbonyl carbon. Interactions of this type are well known (10). An example is shown on page 4 . Such an effect is sterically impossible in a structure such as VII, and the increase in frequency in such a γ amino ketone must be accounted for by simple inductive effects.

It is interesting to note that lycopodine hydrobromide was soluble in cloroform, since in the extraction proceedure for lycopodine an acidic (HCl) aqueous solution of all the extracted material from the plant is shaken with cloroform. Originally, the assumption was made that the alkaloid hydrochlorides would be far more soluble in water than in chloroform. As this is not the case, it is recommended that in future ether should be used to extract the neutral and acidic material from the aqueous acidic solution.

On the assumption that lycopodine had the structure VII (chart I, page 6), an attempt was made to prepare the 1:2 dione IX (chart I page 6) by a series of reactions from both annotinine and lycopodine. The proposed routes are outlined diagrammatically on chart I, page 6 .

Our first efforts towards interrelating lycopodine with annotinine were directed towards the transformation of lycopodine into the corresponding α - diketone, i.e. - $\overset{O}{\overset{\|}{C}} - CH_2 \rightarrow -\overset{O}{\overset{\|}{C}} - \overset{O}{\overset{\|}{C}}--$.

CHART I (PROJECTED SCHEME)



Attempted bromination of lycopodine in glacial acetic acid, containing hydrogen bromide, was complicated by the insolubility of lycopodine hydrobromide in the reaction medium and led to the precipitation of the salt of the starting material. Since lycopodine hydrobromide is moderately soluble in chloroform the bromination was carried out in this solvent. A crystalline product, which proved to be monobromolycopodine hydrobromide, separated in 74 percent yield. The infrared spectrum ($CHCl_3$) showed a band at 1730 $cm.^{-1}$, a shift towards higher frequency of 12 $cm.^{-1}$ as compared to the salt of the starting material. Such a shift is typical of an α - bromo cyclohexanone in which the bromine occupies an equatorial conformation (11). Since the initially formed bromo compound is the axial isomer, the ready epimerisation indicated that a) bromination has occurred at a methylene carbon and b) in the initially formed axial isomer there is at least one 1:3 diaxial bromine: substituent interaction (12). This is illustrated on page 4, where R and R$'$ cannot both be hydrogen.

When the correct structure of lycopodine (XVI, page 4) became known (13), it was realised that the shift of 12 $cm.^{-1}$ towards a higher frequency in the carbonyl absorption (in the infrared) of monobromolycopodine hydrobromide, compared to lycopodine hydrobromide, might be of value

in determining the relative stereochemistry of lycopodine. Assuming that all the cyclohexane rings are present in the chair form, and ignoring the configuration of the carbon bearing the methyl group, there are three plausible stereoisomeric forms, XVII, XVIIA, and XVIII (shown on page 4) for lycopodine, and a consideration of the infrared spectral data of monobromolycopodine tends to eliminate structure XVIII as a possibility, since in structure XVIII there would be no 1:3 diaxial bromine: substituent interactions. Further work in this department indicates that structure XVIIA is the correct one.

Further attempts to prepare a polybromolycopodine, using larger volumes of chloroform led to monobromolycopodine and an intractable oil, which could not be induced to crystallize and which decomposed on exposure to air.

Since the possibility existed that dibromolycopodine was formed, but that it was very difficult to purify or was unstable, an attempt was made to prepare a polybromolycopodine and then to hydrolyse it directly with alkali.

Rather than use lycopodine, of which little was available, as the starting material for the preparation of polybromolycopodine, the residues from the previous bromination experiments were used. As in the case of lycopodine hydrobromide the residues were insoluble in glacial acetic acid and hence were dissolved in chloroform. A brown oil was

obtained after treating the residues with bromine and then potassium hydroxide. In an attempt to purify the oil, it was chromatographed, but this proceedure did not yield any crystalline compounds. However, the spectral properties of the main fraction from the chromatogram are of interest, since peaks at 1665, 1632, and 1605 cm.$^{-1}$ in the infrared ($CHCl_3$ solution) and maximal absorption at 332, 315 and 257mμ, in the ultraviolet indicate the presence of unsaturation.

Kornblum and co-workers (14) have shown that aliphatic monobromo compounds can often be converted to the corresponding ketone by dissolving the bromo compound in dimethyl sulphoxide, and leaving at room temperature. The use of this reagent on monobromolycopodine was particularly appealing, since under these mild conditions of temperature and reagent there seemed little possibility of polymerization and undesirable by-product formation taking place. Either a high recovery of starting material or a high yield of product was expected, since the work up would merely consist of adding water to the dimethyl sulphoxide solution to decompose the solvent, basifying the resulting aqueous solution and extracting with chloroform. Upon performing the experiment, the only product was a yellow viscous oil, which although exhibiting peaks in the infrared spectrum (chloroform solution) at 1705 and 1640 cm.$^{-1}$, which could be indicative of the enol form of a 1:2 dione, unfortunately could not be converted to a crystalline form.

Selenium dioxide is an oxidant that will frequently oxidise a $-CH_2-CO-$ group to a $-CO-CO-$ group, although the reagent also effects allylic oxidations. Since there are no allylic hydrogens present in lycopodine, possible byproducts would only be produced by oxidation of the initially formed 1:2 dione. Hence if oxidation does occur, it should be possible to isolate the 1:2 dione. The oxidation was attempted with dioxane as solvent, but only unchanged lycopodine was obtained (89% recovery). The oxidation has since been repeated in this department (15), using ethanol, and also acetic anhydride as solvents. With ethanol, lycopodine was again recovered and with acetic anhydride the enol acetate of lycopodine was obtained.

In a further attempt to prepare the 1:2 dione from lycopodine, lycopodine was treated with ethyl formate under basic conditions in the hope that a monohydroxymethylene derivative of lycopodine would be formed, since ozonolysis of the hydroxymethylene group, which is always formed α to a ketone by the attack of the ethyl formate on the activated α keto hydrogens, would then yield the required 1:2 dione. However, only intractable brown oils were obtained.

Since lycopodine does not readily yield a pure hydroxymethylene derivative, there is not much liklihood that either benzylidene or furfurylidene derivatives are obtainable, since both the benzylidene and the furfurylidene groups are considerably larger than the hydroxymethylene group and hence are much more susceptible to steric hindrance. Lycopodine was therefore not reacted with either benzaldehyde or furfuraldehyde

At this point in the work on lycopodine, the only crystalline derivatives of lycopodine that had been successfully prepared were lycopodine hydrobromide and monobromolycopodine hydrobromide. Rather than start work that involved the use of more lycopodine, it was considered more satisfactory if one or both of these derivatives could be used to prepare the 1:2 dione. Monobromolycopodine hydrobromide had already been treated with dimethyl sulphoxide, but it was then decided to attempt the alkaline hydrolysis of monobromolycopodine hydrobromide in order to effect the conversion of the -CO - CH (Br)- group to the - CO - CH (OH) - group, which could then be oxidised to the 1:2 dione. The reaction was tried but was no more successful than any of the previous reactions with lycopodine, since several intractable brown oils were obtained. It is possible that elimination of the bromine as hydrobromic acid, rather than hydrolysis occurred, since the oils exhibited peaks in the infrared (chloroform solution) at 1642 and 1630 $cm.^{-1}$, that are indicative of carbon - carbon double bond unsaturation and a peak in the ultraviolet at 278$m\mu$, which also suggests that a rearrangement may have occurred.

A Zimmermann reaction (16) was then carried out with lycopodine. This is a colour test for the presence of a $-CO - CH_2 -$ group in ketosteroids. The reagent consists of ethanolic 1% meta dinitrobenzene and aqueous potassium

hydroxide). A positive reaction would have definitely indicated the presence of 2 α hydrogens. However, a negative reaction was obtained, which was virtually meaningless, since it is possible that the test is sensitive to steric effects.

Since the preparation of the 1:2 dione from lycopodine had not been accomplished and it seemed unlikely that further work would readily yield the dione, it was decided that a different mode of attack on the problem of interrelating lycopodine and annotinine might prove more successful. Whereas the scheme for the interconversion of lycopodine and annotinine to a common intermediate that is outlined on chart I involves oxidative pathways, it seemed possible at this point, that reductive pathways could well prove more profitable.

Essentially a reductive correlation of annotinine and lycopodine consists of the deoxygenation of annotinine and lycopodine. The deoxygenation of annotinine can be accomplished in such a manner that four stereoisomeric carboamines, XXIII, XXV, XXVI, and XXVII (chart II, page 17) are obtained.

This deoxygenation proceedure has two advantages over the previously mentioned oxidative interrelation, since the conversion of lycopodine to one of the carboamines would be accomplished even if a) the keto group of lycopodine was not in the position (in ring B) indicated

in structure VII (chart I, page 6) and b) the stereochemistry of lycopodine is not that indicated in structure VII. It has the additional advantage that deoxygenation of any other Lycopodium alkaloid would immediately show whether it possessed the carbon-nitrogen skeleton of annotinine.

The deoxygenation of lycopodine appeared to be a relatively simple matter since it would only involve a Wolff Kischner reduction of lycopodine and in fact it was later shown, in this department (I7) that this was indeed the case, the reaction proceeding in high yield.

The deoxygenation of annotinine is not such a simple matter since there are essentially three oxygen containing functional groups that have to be removed, a) the epoxide ring, b) the lactonic carbonyl and c) the potential hydroxyl group of the lactone ring. There are several routes by which the oxygens might be removed but the one which initially commended itself is shown on chart II (page I7). This is both a longer and less economical route than is necessary, but concurrent with the work on lycopodine the series of reactions shown on chart I (page 6) for the conversion of annotinine to a I:2 dione was initiated and the sixth compound in the series, methyl epidesoxidoannotinate lactam VI prepared. Since the epoxide ring of annotinine was not present in methyl epidesoxidoannotinate lactam, use was made of this ester in the attempted deoxygenation of annotinine, as shown on chart II (page I7).

Before dealing with the attempted removal of the hydroxyl and carbomethoxy groups from methyl epidesoxidoannotinate lactam the preparation of methyl epidesoxidoannotinate lactam will be discussed. The starting material, annotinine I (chart I, page 6) was extracted from *Lycopodium annotinum* L. by the method of Manske and Marion (9). It is interesting to note that 90g. of annotinine were obtained from 65kg. of plant or I.4g. of annotinine per kg. of plant compared to the literature value of 0.5g. per kg.

The locality of growth of the plant, the proportion of roots present in the picked plant, the time of year when the plant is picked and slight variations in the extraction proceedure are just a few of the factors that could influence the yield of alkaloids obtained from a particular sample of plant. However, since a rigorous study of the variations of the yield of alkaloids from, *Lycopodium annotinum* L., with the above factors, has never been performed, the reason, or reasons, for our high yield is not known.

Oxidation of annotinine by potassium permanganate readily yielded annotinine lactam II, (chart I, page 6), identified by its infrared spectrum and melting point, but the yield (60%) was rather discouraging. The reason for the fairly low yield is probably that the remainder of the annotinine is converted to the amino acid XIX (see page 20). An attempt was made to recover the amino acid by continuous extraction with ether of the aqueous residues at

pH 1 but the amino acid was not obtained.

The conversion of annotinine lactam into annotinine chlorohydrin lactam, III, (chart I, page 6) was accomplished by refluxing a solution of annotinine lactam in concentrated hydrochloric acid. The yield (89%) of the chlorohydrin, which was identified by its infrared spectrum and its melting point, was good.

The next reaction, which was the dehydration of annotinine chlorohydrin lactam by phosphorus oxychloride proceeded cleanly and in fairly high yield (76%) to give anhydroannotinine chlorohydrin lactam which was identified by its infrared spectrum and its melting point.

Hydrogenation of anhydroannotinine chlorohydrin lactam at room temperature, using platinum as a catalyst, gave crystalline desoxidoannotinine lactam, V, in 70% yield.

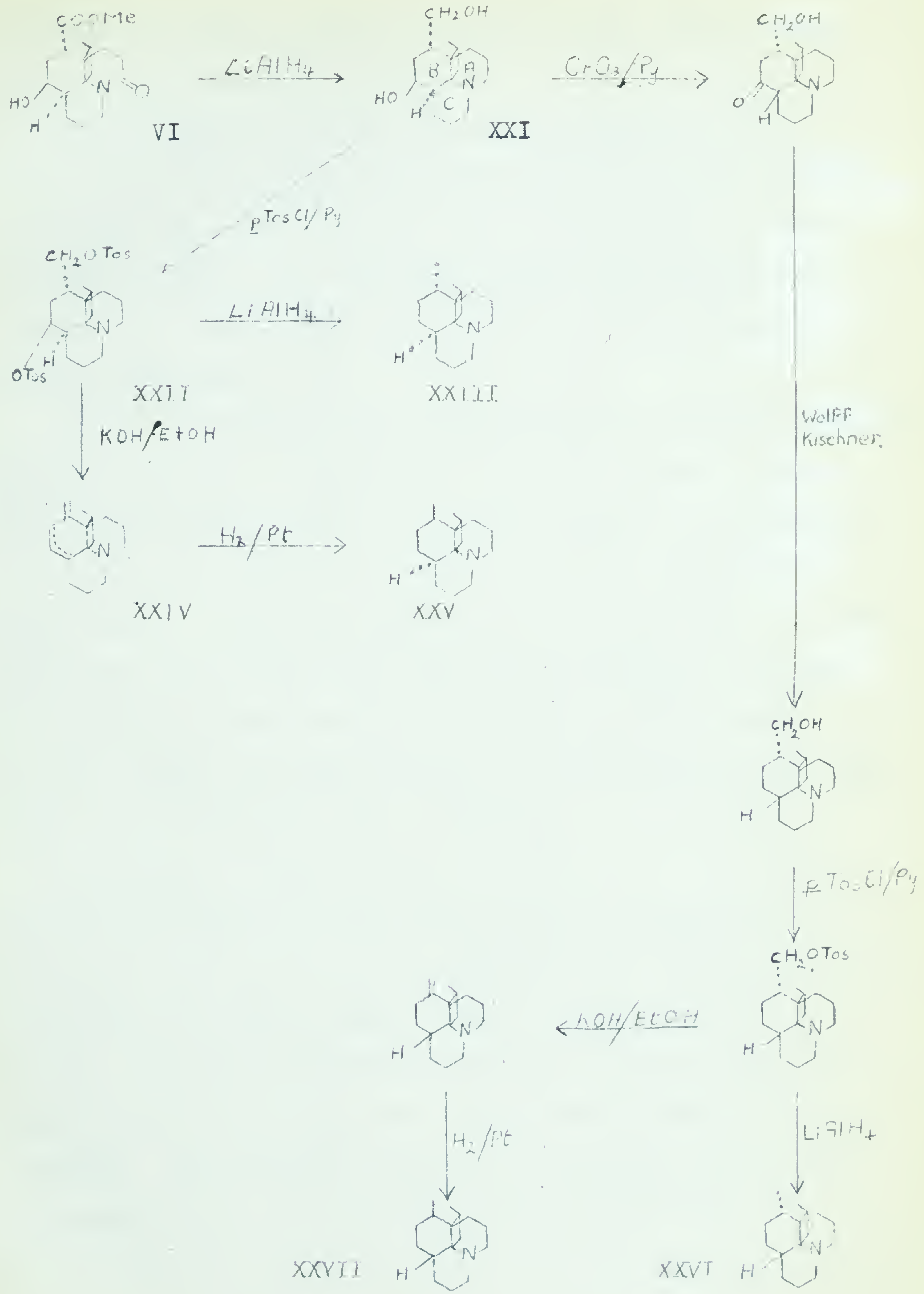
Treatment of desoxidoannotinine lactam, with methanolic potassium hydroxide opened the lactone ring and yielded the required methyl _epi_desoxidoannotinate lactam, identified by its infrared spectrum and melting point, but in low yield (47%). It had been hoped that the lactone ring of desoxidoannotinine lactam would be opened exclusively by methoxide ions to initially yield the unepimerised hydroxy lactamic ester which would then epimerise under the basic conditions present, to yield the required

epimeric ester. However, the lactone ring was also opened by the hydroxide ions present, since some desoxidoannotinic acid lactam XX (see page 20), identified by its infrared spectrum and melting point, was also obtained (40% yield).

In order to improve the overall yield of methyl _epi_desoxidoannotinate lactam an attempt was made to convert all the desoxidoannotinic acid lactam to the epimerised ester by treating the acid with diazomethane and then potassium hydroxide in methanol. Further methyl _epi_desoxidoannotinate lactam (62% yield) was obtained together with a little of the non-epimerised ester, methyl desoxidoannotinate lactam, identified by its infrared spectrum and melting point, (I7.5% yield). Hence the overall yield of methyl _epi_desoxidoannotinate lactam VI (chart I, page 6) was 72%.

The series of reactions by which it was hoped to attain the deoxygenation of methyl _epi_desoxidoannotinate lactam VI is shown on chart II, page I7. It will be seen that the first step is the removal of the lactamic carbonyl group and the reduction of the carbomethoxy group to a primary alcohol, by lithium aluminium hydride. The reduction did not proceed when the reaction was performed in absolute ether and was incomplete when the higher boiling benzene was used. However, lithium aluminium hydride is only sparingly soluble in benzene and the

CHART II (PROJECTED SCHEME)



reaction proceeded in 95% yield when a large excess of hydride in ether-benzene was used.

The initially obtained desoxidoannotininediol XXI was a colourless, viscous oil which crystallised from anhydrous ether as yellow crystals, which decomposed rapidly on exposure to air. Both the hydrochloride and hydrobromide, however, were stable and the compound was characterized as the salt form.

The hydrochloride was treated with p-toluenesulphonyl chloride in pyridine. A crystalline tosylate, either in the form of the free base or as the salt, could not be obtained but the oily product appeared to be a monotosyl derivative, since it exhibited peaks in the infrared that were characteristic both of hydroxyl and tosylate groups. Although the tosylation was repeated several times, using longer reaction times and larger excesses of p-toluenesulphonyl chloride, a ditosylate was never isolated.

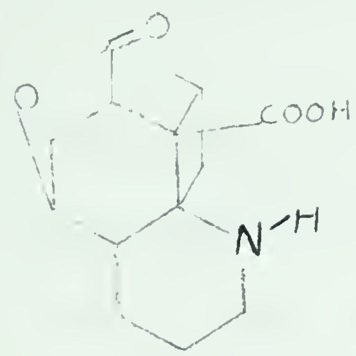
Attempts to eliminate p-toluenesulphonic acid from the monotosylate with refluxing pyridine, methanolic potassium hydroxide and dimethyl sulphoxide led to the recovery of starting material. However, treatment with refluxing ethanolic potassium hydroxide yielded a brown oil which showed hydroxyl but not tosylate bands in the infrared. The compound could not be obtained crystalline and since only a few milligrams were obtained was not

analysed or further investigated.

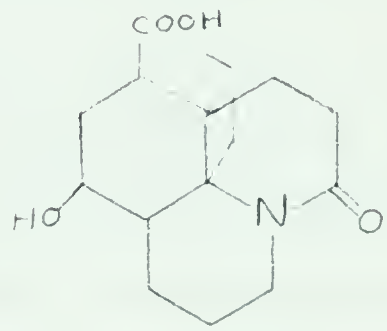
The difficulty with which the p - toluenesulphonic acid eliminated indicates that tosylation of the primary, rather than of the secondary hydroxyl group in desoxido-annotininediol XXI (chart II, page 17) occurred, since a secondary tosylate should readily eliminate under the conditions first used.

The low overall yield in the sequence from annotinine to the diol XXI led us to seek other routes for the preparation of the four carboamines.

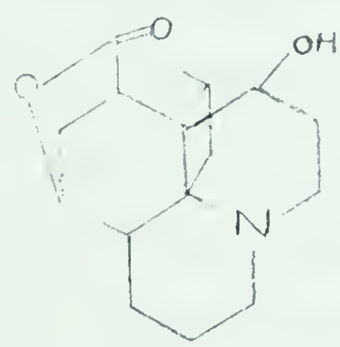
Basically, three oxygen functions have to be removed from annotinine, I (chart I, page 6), namely the epoxide ring, the lactonic carbonyl and the potential hydroxyl group of the lactone ring. A reaction sequence leading through the triol XXXVI (see page 20) which can be prepared by hydride reduction of annotinine, did not seem attractive since both the primary hydroxyl and the secondary, ring A hydroxyl are sterically very hindered and we first turned our attention towards the selective removal of the epoxy oxygen. It should be mentioned at this point that, as is often the case when working with relatively inaccessible naturally occurring substances, possible reaction sequences involving a large number of steps often must, for practical reasons, be avoided. Wittig and Haag (19) have shown that in many



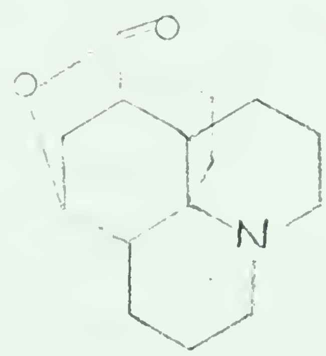
XIX



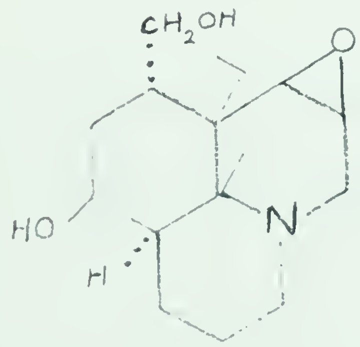
XX



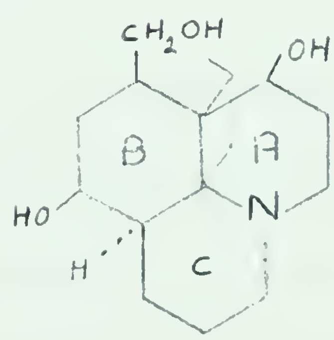
XXIX



XXVIII



XXXV



XXXVI

cases an epoxide may be transformed to the corresponding olefin by treatment with triphenylphosphine. Initially, annotinine and triphenylphosphine were refluxed in a high boiling inert solvent, but annotinine was recovered unchanged, just as it was when fused mixtures of annotinine and triphenylphosphine were heated together in a sealed tube at temperatures of up to 270°.

MacLean (20) reports that hydrogenolysis of annotinine removes the epoxy-oxygen to yield a small quantity of desoxidoannotinine XXVIII (see page 20) and hydroxydesoxidoannotinine XXIX (see page 20) is also produced. Both of these compounds would have been of value had we obtained them, but although the hydrogenolysis was performed under widely divergent conditions of temperature and pressure we were unable to duplicate the results of previous workers.

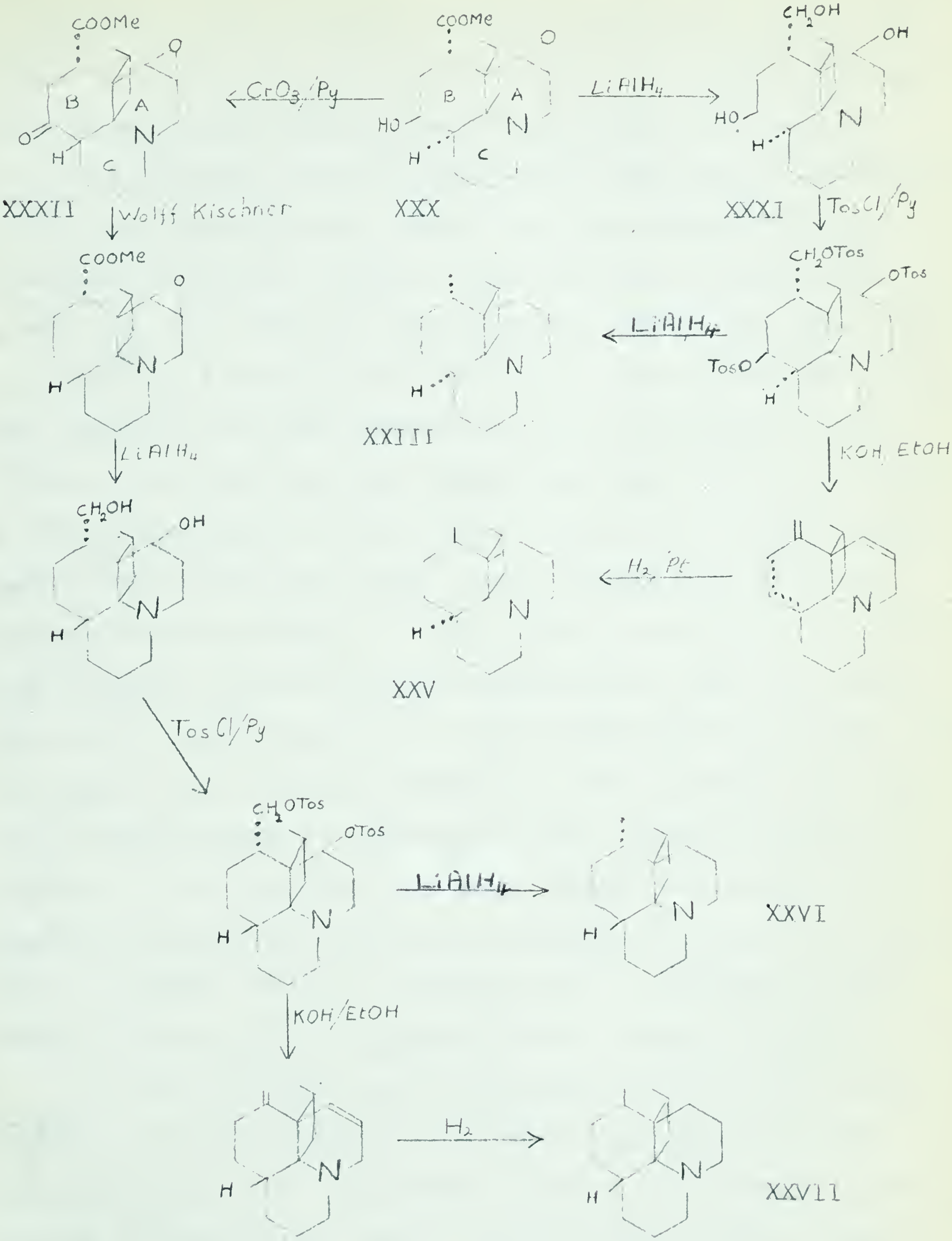
The third and final attempt at removing the epoxide involved treating annotinine with lithium and ethylamine. The product expected was again hydroxydesoxidoannotinine XXIX (see page 20) but the reaction failed to yield any characterisable products.

The third series of reactions for the deoxygenation of annotinine is shown diagrammatically on chart III, page 22.

The first step of the series requires that the lactone ring of annotinine be opened to yield an hydroxy epimerised methyl ester, in which the epoxide ring is still closed, since if the epoxide opened, we would have four oxygen functions to remove.

The required methyl _epi_annotinate had previously been

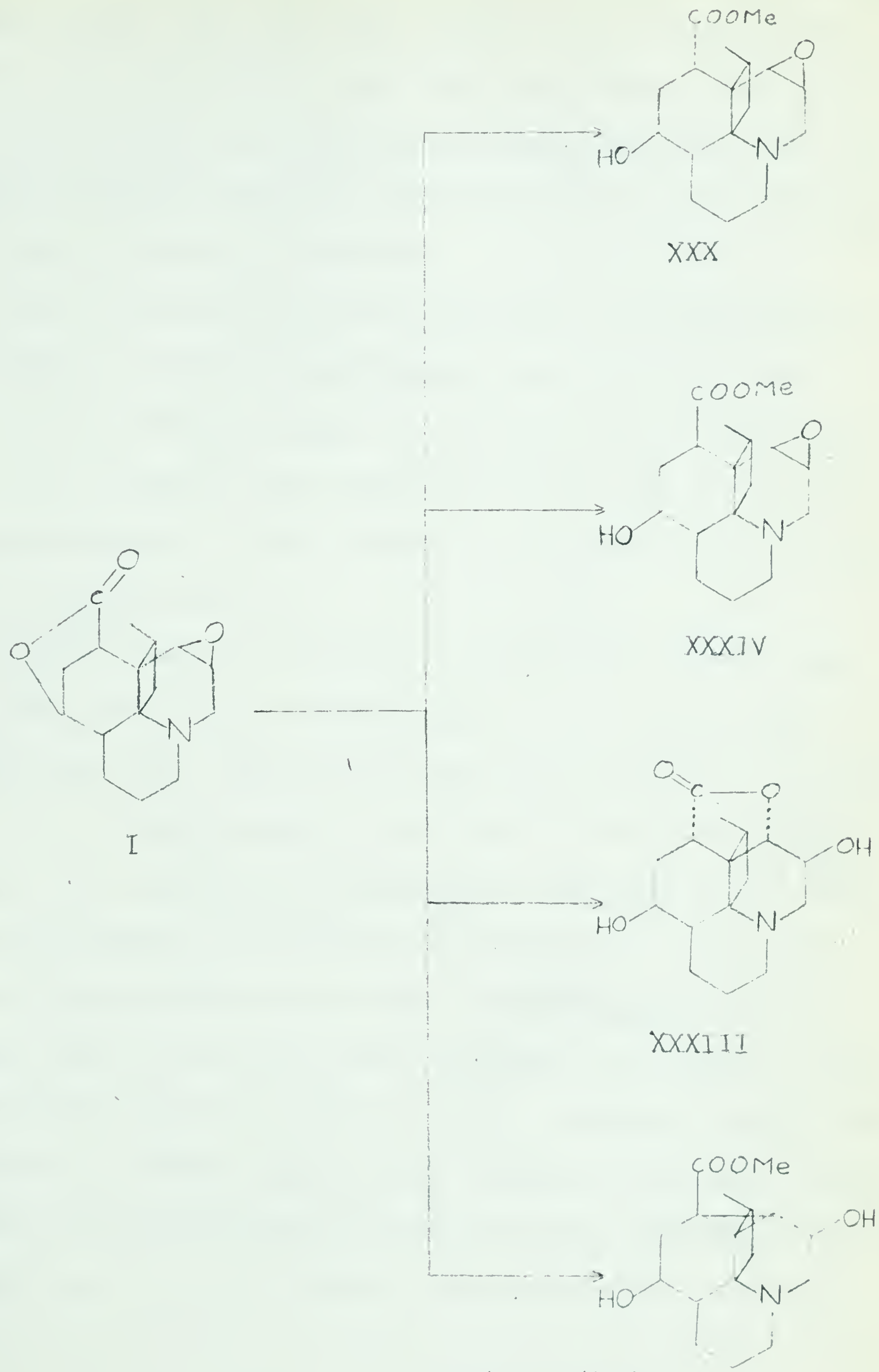
CHART III (PROJECTED SCHEME)



prepared in 75% yield by Marion and co-workers (38) by treatment of annotinine with methanolic potassium methoxide. We have been unable to obtain consistent results with this reaction and have isolated in varying yields (see experimental), four products (chart IV, page 24), methyl *epi*annotinate XXX, methyl annotinate, XXXIV, annotinate hydrate, XXXIII and a new compound $C_{17}H_{25}O_4N$ formulated as XXXVII, (chart V, page 28) which we shall refer to as ester M. The derivation of structure, XXXVII, for ester M will be discussed in detail later. The use of sodium methoxide rather than potassium methoxide seemed to have little effect on the course of the reaction. The methanol used was dried over magnesium and a determination of the water content by the Karl Fischer method showed less than 0.01% of water present. It is likely that annotinine hydrate is formed during the work up from methyl *epi*annotinate, since infrared measurements on the crude product obtained by simply evaporating the solvent from the reaction mixture showed no γ-lactonic absorption in the infrared, and since methyl *epi*annotinate is transformed into annotinine hydrate on prolonged contact with aqueous base. Methyl annotinate is an intermediate in the formation of methyl *epi*annotinate. The mechanism of formation of these compounds will be discussed in more detail in connection with the structural studies on ester M.

It was found that the carbomethoxy group of methyl *epi*annotinate was easily reduced to a primary alcohol by lithium aluminium hydride but the epoxide ring was untouched and even the use of high boiling ether, tetrahydrofuran, and a large

CHART IV



. Ester M, XXXVII

excess of lithium aluminium hydride did not yield the required triol XXXI (chart III, page 22). Since the product of the reduction, the diol epoxide XXXV (see page 20) was analysed as its hydrochloride and its hydrobromide, the free base was regenerated from the salts to ascertain that no reaction had occurred during the salt formation.

It will be remembered that only one of the hydroxyl groups of the desoxidoannotininediol could be tosylated. Since the two hydroxyl groups of the diol epoxide XXXV (see page 20) are in exactly the same position as the two in desoxidoannotininediol it was interesting to study their accessibility to acetylation.

Hence the ease of acetylation of the diol epoxide was compared with the ease of acetylation of annotininetriol XXXVI (see page 20). One would expect that both of the hydroxyl groups in the diol epoxide would readily acetylate, since on a model they appear to be relatively unhindered, but that complete acetylation of annotininetriol would not occur, since the primary hydroxyl is badly hindered and the secondary hydroxyl in ring A is also hindered. In neither case was an analytically pure solid isolated. However the infrared spectra did not exhibit any hydroxy peaks for the diol epoxide acetate but the annotininetriol acetate did, and both acetates exhibited bands at the usual characteristic acetate

frequencies which indicated that both the hydroxyls in the diol epoxide had been acetylated.

At this point it was unequivocally shown by chemical analysis and by nuclear magnetic resonance studies (17) that lycopodine possessed only one carbon - methyl group. Therefore, lycopodine could not have the structure VII (chart I, page 6) and hence the urgency to obtain the four deoxygenated compounds from annotinine was no longer present. It was also obvious from the difficulty experienced in tosylating desoxidoannotininediol and in reducing the epoxide ring of methyl epiannotinate that the route to the four deoxygenated compounds from annotinine was more complicated than had been anticipated and our energies were directed towards the solution of the problems discussed below.

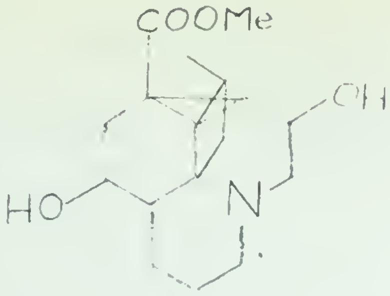
STRUCTURE OF ESTER M

Throughout this discussion on ester M, ester M will be shown diagrammatically as its assigned structure XXXVII (chart V, page 28). Ester M is isolated as a white solid that is insoluble in all common cold organic solvents except glacial acetic acid, but is soluble in refluxing methanol or ethanol. It can be purified either by crystallisation or by sublimation as white rhomboids. The infrared spectrum (nujol) exhibited absorption at 3440 $cm.^{-1}$ which indicated the presence

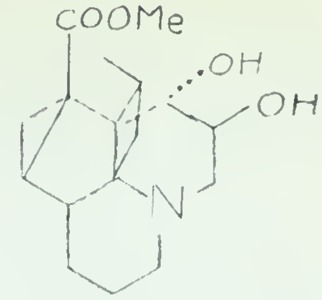
of one or more hydroxyl and/or NH groups; 2600 - 2800 cm.$^{-1}$, and 1724 cm.$^{-1}$, which was rather low for an ester and rather high for an acid. The fingerprint region was distinctly different from that of methyl <u>epi</u>annotinate. The absorption between 2600 and 2800 cm.$^{-1}$ suggested that ester M was a carboxylic acid. Analysis showed that the molecular formula was $C_{17}H_{25}O_4N$ and that there was one -O-Me present. The methoxy group could have entered the molecule in three ways. Either (i) by attack on the lactonic carbonyl of annotinine to yield an ester or (ii) by opening the lactone with alkyloxy fission or(iii) by opening the epoxide ring.

Case(ii) would lead to the intermediate XXXVIII (chart V, page 28), which possesses a β carboxylate ion. The carboxylate ion would not be expected to epimerise since the intermediate state XXXIX (chart V, page 28) for epimerisation would not be expected to form at all readily. Hence underside attack by the carboxylate ion of XXXVIII on the β epoxide is not possible since the carboxylate ion cannot epimerise and so the only feasible structure arising from XXXVIII is the acid XL (chart V, page 28). However it was readily shown that ester M (which is insoluble in aqueous base) was not an acid since an ethanolic solution of ester M was readily hydrolysed by barium hydroxide to an amino acid M XLI (chart V, page 28), which analysed correctly as $C_{16}H_{23}O_4N$ and was readily reconverted to

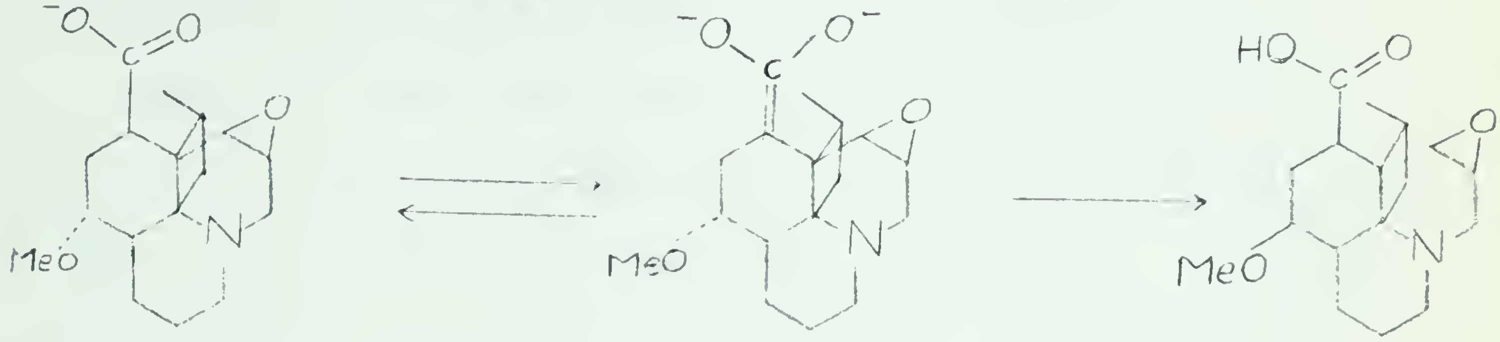
CHART V



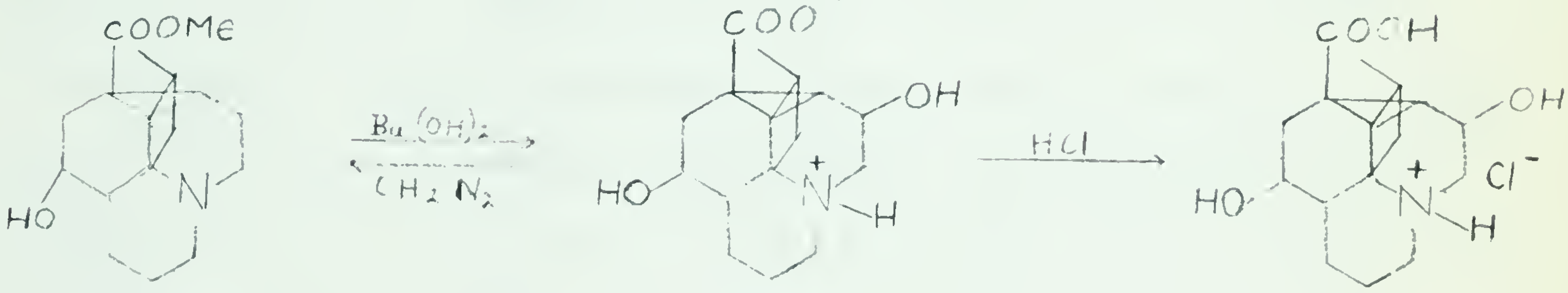
XXXVII



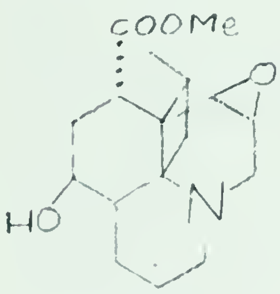
IL



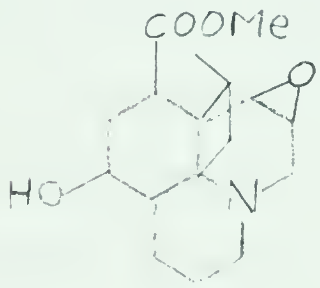
XXXVIII XXXIX XL



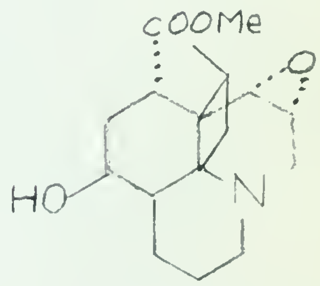
XXXVII XLI XLII



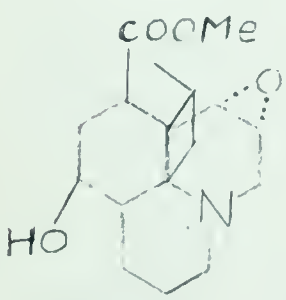
XXX



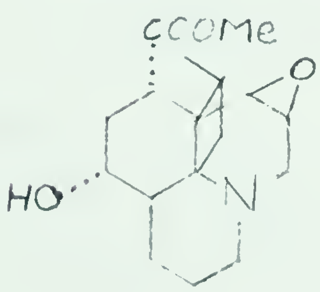
XXXIV



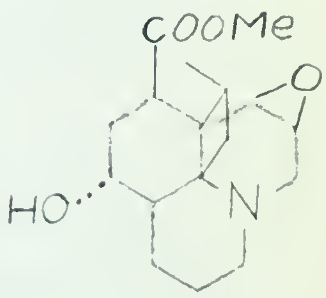
XLIII



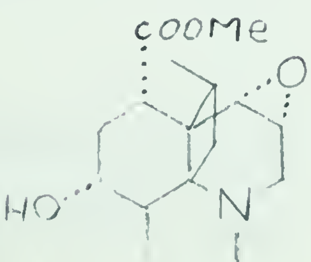
XLIV



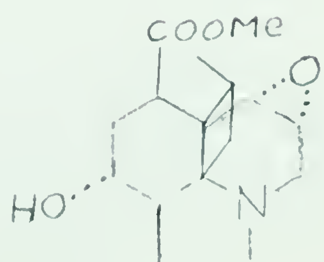
XLV



XLVI



XLVII



XLVIII

to ester M by treating a methanolic solution of the acid with diazomethane. (The hydrolysis will be dealt with more fully below.)

Structures arising from case (iii) were eliminated since ester M had been shown to be a methyl ester.

From the above data and the origin of ester M there were only a limited number of likely structures for ester M that could arise from case (i). Thus there was methyl epiannotinate XXX (chart V, page 28) and its seven epimers, that are epimeric at one or more of the hydroxyl, carbomethoxy and epoxide centres. These eight structures, XXX, XXXIV, and XLIII to XLVIII inclusively, are shown on chart V, (page 28). The two other structures upon which I shall later comment in detail are XXXVII and IL (chart V, page 28). Structures XXX(methyl epiannotinate and XXXIV (methyl annotinate) were readily eliminated as possibilities since they are known compounds and are distinctly different in melting point and infrared spectra. Of the remainder of the compounds, all contain an epoxide ring with the exception of XXXVII and IL which instead contain cyclopropane rings.

Work was then performed to eliminate some of the possible structures for ester M that are listed on chart V (page 28).

Methyl epiannotinate XXX (charts IV and V, pages 24 and 28) is readily converted under acidic conditions to annotinine

hydrate XXXIII (chart IV, page 24), Ester M was therefore dissolved in glacial acetic acid to see whether a similar isomerisation would occur. After 26 hours the glacial acetic acid was evaporated to yield unchanged ester M which showed that ester M did not isomerise under mildly acidic conditions. A solution of ester M in dilute hydrochloric acid was then refluxed for 10 hours. The acidic solvent was removed azeotropically with benzene and 98% ethanol, to yield acid M hydrochloride. (The salt was identified by its melting point and its infrared spectrum (nujol)).

The fact that isomerisation had not occurred eliminated the structure XLV (chart V, page 28), as a possible structure and the fact that a chlorohydrin was not produced meant that either an epoxide ring was not present or that it was sterically hindered. Forcing acidic conditions were next used in case a hindered epoxide was present. A methanolic solution of ester M was saturated with hydrogen chloride and was then refluxed under anhydrous conditions for 14 hours. Acid M hydrochloride was again obtained (83% yield).

The possibility did exist that acid M hydrochloride was actually acid M chlorohydrin hydrochloride, but this supposition was disproved in two ways. Firstly ester M was converted to ester M hydrochloride LI (see page 32) under the same conditions as acid M was converted

to acid M hydrochloride. Ester M hydrochloride was then reconverted to ester M by the action of ammonium hydroxide on the salt which showed that under these conditions ester M chlorohydrin hydrochloride was not produced.

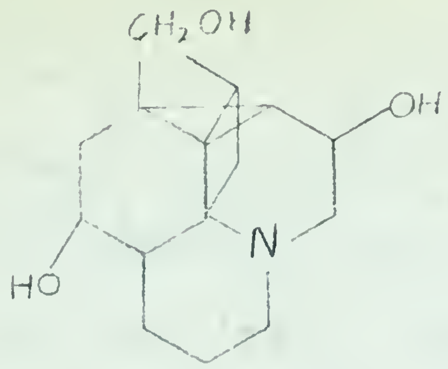
Acid M hydrochloride was not reconverted to the acid M since the resulting amino acid would not be readily isolated.

Secondly acid M hydrochloride analysed as $C_{16}H_{24}O_4Cl$ whereas the chlorohydrin hydrochloride would contain two chlorine atoms. It was therefore considered that there is not an epoxide ring in ester M.

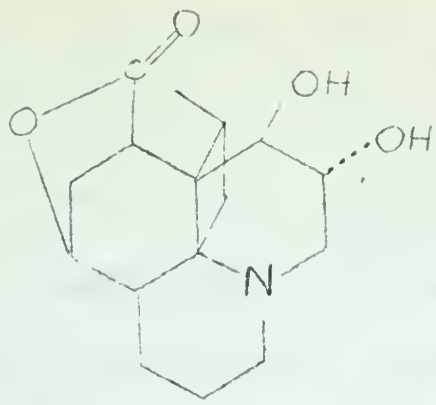
A cyclopropane ring that is α to a ketone readily opens if it is treated with a strong acid. The mechanism of the process is shown on page 32. You will note that the mechanism involves the production of an enol intermediate. Such an intermediate is possible, but not probable, if the cyclopropane ring is $\alpha;\beta$ to an ester rather than a ketone. The mechanism would then be as shown on page 32.

It is not unreasonable to suppose that the absolute methanolic hydrogen chloride would not open the cyclopropane ring of structures XXXVII and IL (chart V, page 28)

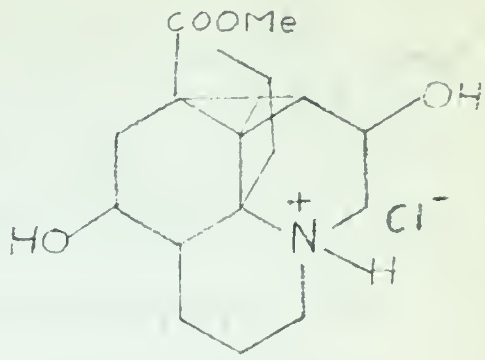
We shall now return to the basic hydrolysis of ester M by barium hydroxide. Two acids (M and N) were obtained, which at first appeared to be chemically different. Acid M XLI (chart V, page 28) was crystallised from water - benzene - 98% ethanol as white prisms m.p. 329° (uncorr.) I.R. (nujol)



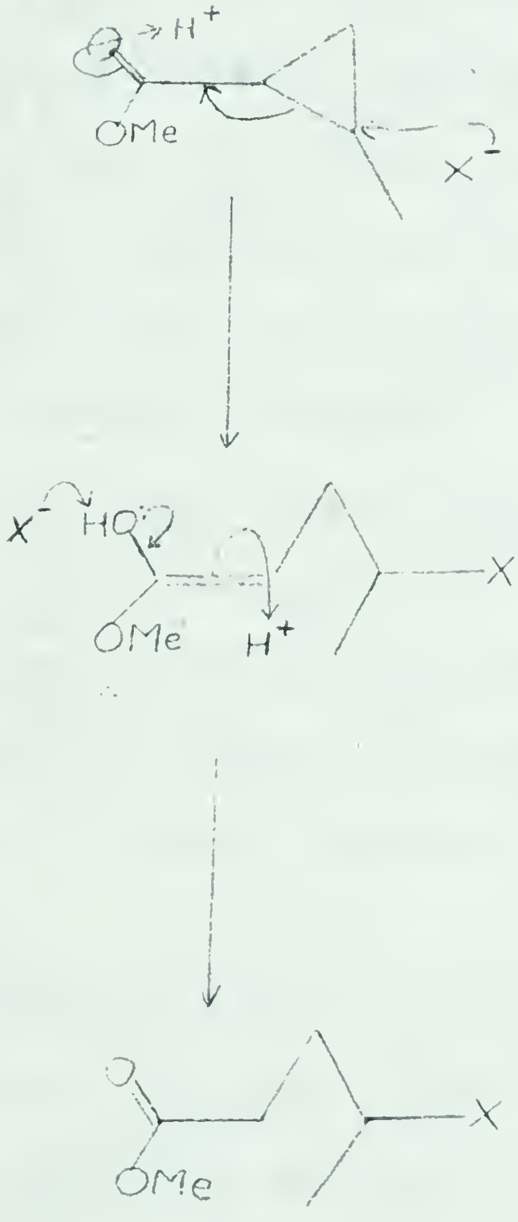
L

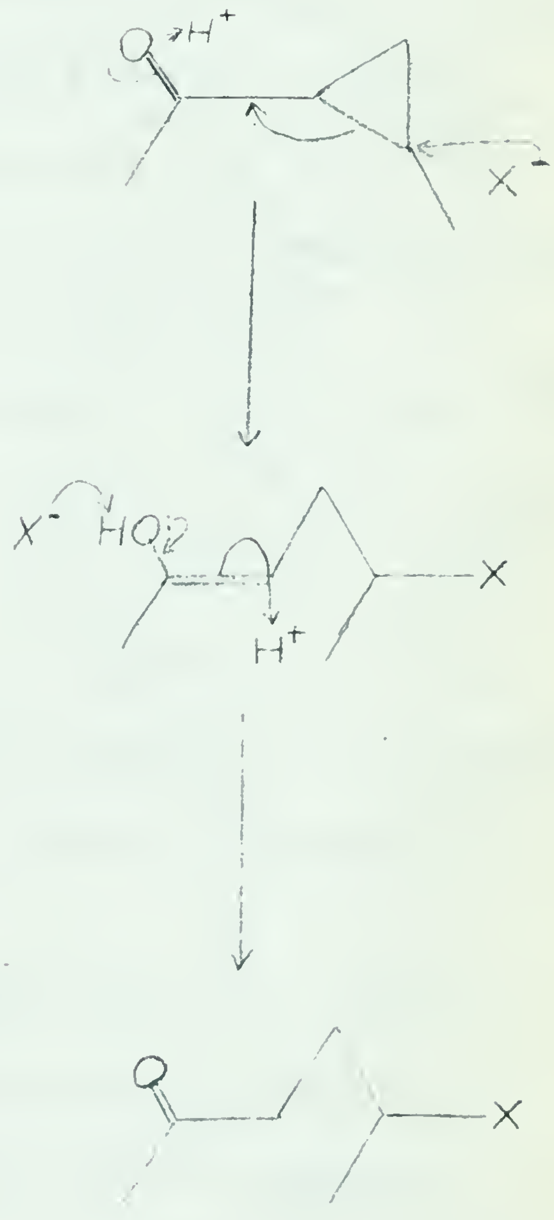


LX



LI





3570, 3510, 2500 - 3000, 1658 and 1565 $cm.^{-1}$. The bands at 3570 and 3510 $cm.^{-1}$ indicated that one or more hydroxyl groups were present, while the broad band at 2500 - 3000 $cm.^{-1}$ could well have been due to $-\overset{+}{N}-H$ stretching and the peak at 1565 $cm.^{-1}$ due to the carbonyl stretching of a carboxylate ion. Hence the infrared spectrum indicated that acid M existed as the Zwitter ion XLI (chart V, page 28) and the peak at 1658 $cm.^{-1}$ indicated that a double bond was present. However, it was easily shown that there was not a carbon - carbon double bond since an ethanolic solution of acid M did not yield a colour with tetranitromethane, and also the analysis only differed from that of ester M by a CH_2 unit.

The acid N was obtained in a 26% yield, as compared to a 53% yield for the acid M. Crystallisation of acid N from methanol - acetone yielded white prisms m.p. 272° - 302° (dec.) I.R. (nujol) 3480 $cm.^{-1}$ (OH), 3240 $cm.^{-1}$ (OH or $-\overset{+}{N}-H$), 2400 - 2500 $cm.^{-1}$ ($-\overset{+}{N}-H$) and 1585 $cm.^{-1}$ ($-COO^{-}$). The finger-print of the spectrum of acid N bore little resemblance to that of acid M.

The hydrochlorides of acid M XLII (chart V, page 28) and acid N were prepared by the addition of concentrated hydrochloric acid to solutions of the acids in organic solvents. Removal of the solvents and crystallisation of the residues from methanol - acetone in each case, yielded white stars. Acid M hydrochloride and acid N hydrochloride

both melted at 308° with decomposition and exhibited identical infrared spectra (nujol) 3480 $cm.^{-1}$ (OH), 3300 $cm.^{-1}$ (OH or $-N^{+}-H$), 2700 $cm.^{-1}$ ($-N^{+}-H$), 2520 $cm.^{-1}$ ($-N^{+}-H$), 1719 $cm.^{-1}$ (-COOH) and 1632 $cm.^{-1}$.

Since both acids yielded the same salt with a peak in the infrared (nujol) at 1632 $cm.^{-1}$, it was thought that a double bond may have been formed in acid N hydrochloride by an acid catalysed process. However, this was soon eliminated as a possibility since the salt analysed as $C_{16}H_{24}O_{4}NCl$, which differs from the ester M hydrochloride by CH_2. Also both acids were readily reconverted to ester M by diazomethane, which precludes that acid M is not structurally identical to acid N and either is a rearranged product of ester M.

The only possible explanation of the infrared spectra of acids M and N is that both the spectra were of nujol mulls. Hence, acid M could exist as a crystalline modification of acid N that exhibits a different spectrum. This supposition could not be proven by the simple expedient of obtaining solution spectra of the two acids, since both acids were insoluble in all of the suitable spectroscopic solvents, including acetonitrile. However, a saturated solution of acid M in water - benzene - ethanol was seeded with acid N and crystals of acid N were obtained, which proved that acid M was a crystalline modification of acid N.

A benzene solution of ester M was reduced with lithium aluminium hydride to triol M, ($\angle$ see page 32)which gradually decomposed upon crystallisation from acetone and so was converted to the hydrochloride by treatment with concentrated hydrochloric acid. The salt was quite stable and analytically pure, white prisms were readily obtained by crystallisation from glacial acetic acid - acetone. The triol hydrochloride analysed as $C_{16}H_{26}O_3NCl\ \frac{1}{3}H_2O$ and exhibited peaks in the infrared (nujol) at 3430 $cm.^{-1}$ (OH), 3330 $cm.^{-1}$ (OH), 3230 (OH or $-N^{+}-H$), 2650 $cm.^{-1}$ ($-N^{+}-H$) and 2600 $cm.^{-1}$ ($-N^{+}-H$), but did not exhibit any carbonyl absorption. The triol was prepared in the hope that a compound of known structure could be reduced to the same triol at a later date. However, a suitable compound was not found and for this reason the analysis was not repeated.

A simple method of distinguishing between the possible structures for ester M which contain one hydroxyl group and an epoxide and the structures XXXVII and IL (chart VI, page 38) which contain two hydroxyls was to determine the number of active hydrogens present in ester M. However, the Zerewitinoff determination was negative. Since, however, the infrared showed hydroxyl absorption, ester M was acetylated with pyridine and acetic anhydride. At 100° , a solid was obtained whose infrared spectrum indicated that it was an hydroxyacetate and at room temperature an O-diacetate

was obtained, as shown by its infrared spectrum and by analysis. This shows that ester M contains two hydroxyl groups, both of which are probably sterically hindered. Hence, either XXXVII or IL is indicated as the structure of ester M.

An attempt was made to lactonise acid M to see whether a relationship could be established between the carboxylic acid group and one of the hydroxyl groups. A mixture of acid M, p-toluenesulphonic acid and benzene was refluxed under the usual lactonising conditions for three days. Lactonisation did not occur and starting material was recovered.

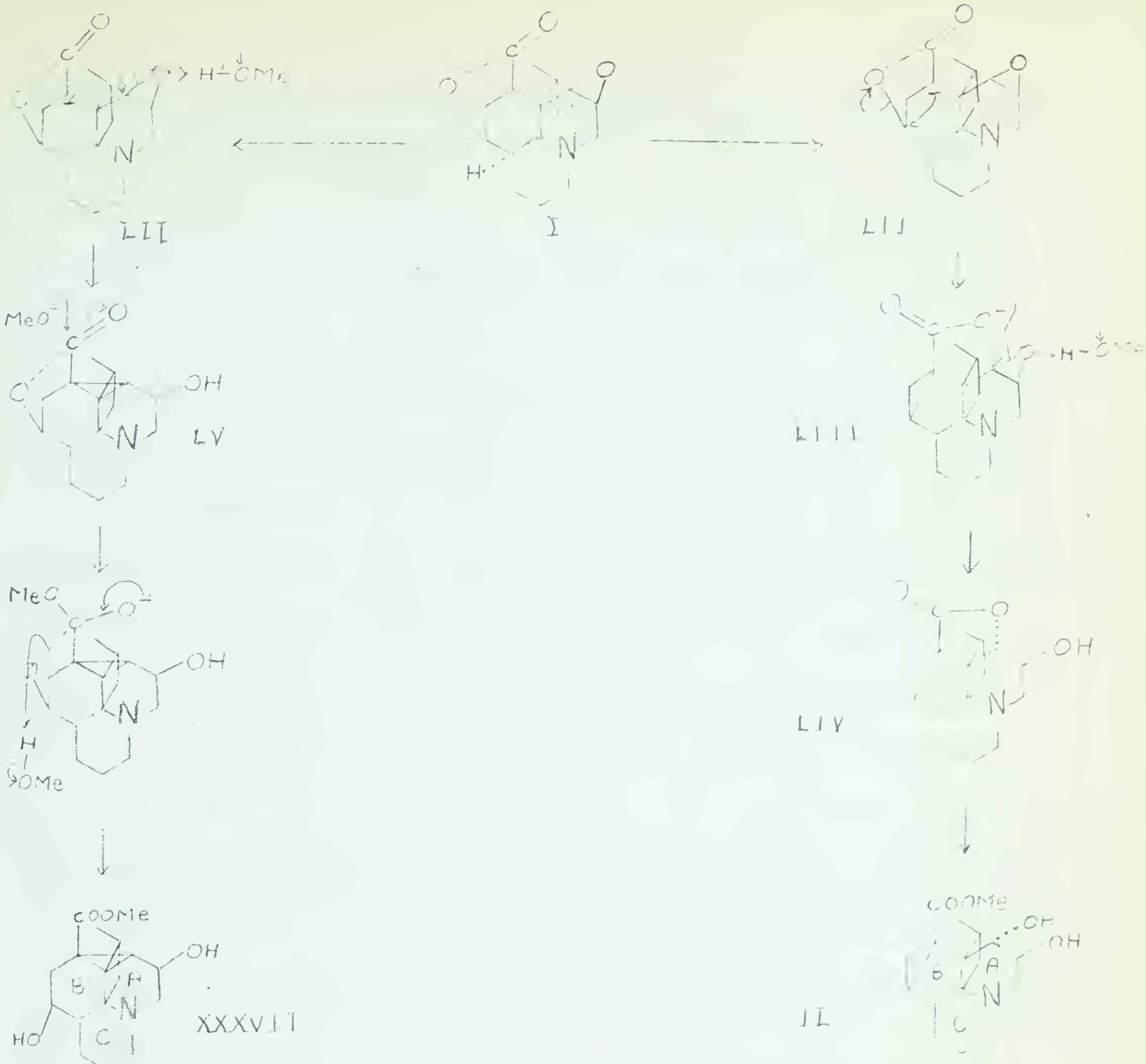
At this point it was clear that the structure of ester M must agree with the following facts. The molecular formula is $C_{17}H_{25}O_4N$, consistent with analytical results obtained with the ester, its acid and the salts of the acid. The compound contains a carbomethoxy group, two hydroxyl groups and a tertiary nitrogen (the basic diacetyl derivative showed no -NH absorption). No evidence could be obtained to support the presence of a double bond and hence the molecule must be pentacyclic. Finally the compound must be derived from annotinine by a mechanistically reasonable pathway. It seemed to us that annotinine is vulnerable to attack by methoxide at two positions, the lactone carbonyl and the carbon α to the lactone carbonyl. Attack

at the carbonyl leads to the formation of methyl annotinate, which can be transformed by attack at the α-carbon in the initially formed ester, XXXIV, to methyl epiannotinate XXX (see chart V, page 28). It should be mentioned at this point that methyl epiannotinate is not an intermediate in the formation of ester M.

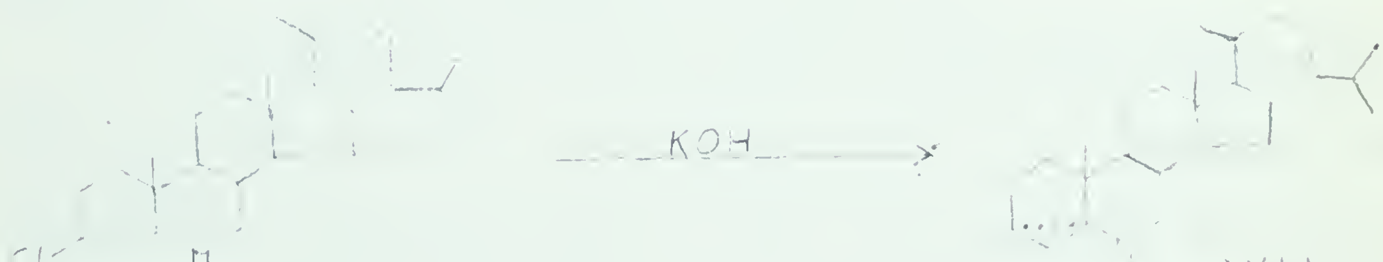
The first step for the formation of both XXXVII and IL is the abstraction of a proton from the carbon atom α to the lactone carbonyl, to yield a carbanion intermediate LII (chart VI, page 38). For the production of IL, the next step is the opening of the lactone ring with alkyloxy fission by the backside attack of the carbanion, to produce a cyclopropane ring and a carboxylate ion LIII (chart VI, page 38). This step is quite plausible, since the lactone ring is badly strained due to its close proximity to the methyl group on the four membered ring. (The alkyloxy fission finds an analogy in the case of a strained β-lactone, which always opens in this fashion. In annotinine the methyl on the four membered ring also hinders the attack of the methoxide ion on the lactonic carbonyl.) The carboxylate ion LIII (chart VI, page 38) then attacks the epoxide ring from the underside to yield the lactonic intermediate LIV (chart VI, page 38) which then opens in the usual manner to yield IL.

The mechanistic route to XXXVII involves the attack of the carbanion on the epoxide ring, to yield the hydroxylactone LV (chart VI, page 38). The lactonic carbonyl is

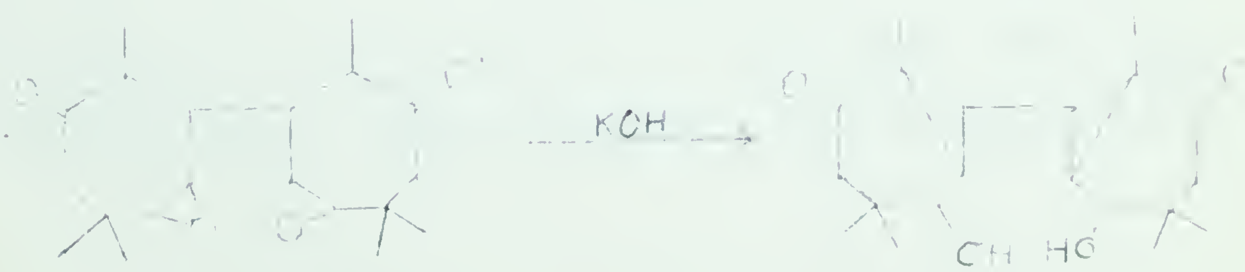
CHART VI



LII I LIII LV LIII LIV XXXVII IL



LVI LVII



LVIII LIX

somewhat hindered towards nucleophilic attack and there are innumerable instances in the literature where a carbanion attack occurs quite readily. In the next and final step, the lactone ring is opened in the usual manner, to yield XXXVII.

There are several analogies in the literature for this type of mechanism, in which a carbanion displaces a group with the formation of a three membered ring. The classical example is Windaus and Dalmer's (21) preparation of hetero-cholestenone LVII (chart VI, page 38) by the action of potassium hydroxide on 3α-chloro-6-ketocholestane LVI. An example which bears an even closer analogy, since it involves the attack of a carbanion on an epoxide ring, to yield a cyclopropane ring is also shown on chart VI. Bucki and Saari (22) converted α-diepoxydieucarvelone LVIII (chart VI, page 38) into an isomeric cyclopropyl ketone LIX (chart VI, page 38).

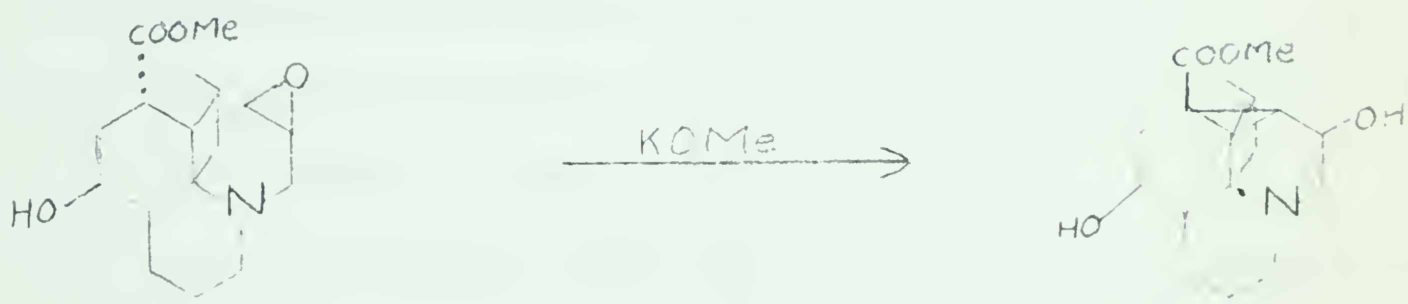
Several attempts were then made to prove the proposed mechanism of the formation of ester M.

If structure IL is the correct structure for ester M, then annotininediol, LX (see page 32) upon treatment with potassium methoxide should yield the compound, LXI (chart VII, page 40) that is epimeric with IL (chart VI). Annotininediol was prepared by the action of refluxing 25% sulphuric acid on annotinine. Initially there was some doubt about the

CHART VII

LXXIII LXI

XXXIII XXXVII



XXX XXXVII

LXII LXIII

product, since neither it nor the hydrochloride melted at the literature temperature for annotininediol and annotininediol hydrochloride. The infrared spectrum (nujol) of the salt showed γ -lactonic absorption at 1781 $cm.^{-1}$ which indicated that the salt could be either annotinine hydrochloride LXII (chart VII, page 40) or annotinine hydrate hydrochloride LXIII (chart VII, page 40). Annotinine hydrochloride and annotinine hydrate hydrochloride were, therefore, prepared but their infrared spectra (nujol) did not bear any resemblance to the spectrum of the other salt. An explanation of the melting point discrepancy was obtained, for it was found that a sample of the salt that had been dried for 16 hours at 130° melted at 290° (lit. 292° - 294°).

The annotininediol hydrochloride was converted to the free base which was then treated with absolute methanolic potassium methoxide. A compound was obtained as a yellow oil that was not annotininediol and whose infrared spectrum did not bear any resemblance to the spectrum of ester M or acids M or N. Before it had been shown that ester M did not possess an epoxide ring, it was possible that ester M was an intermediate in the formation of methyl <u>epi</u>annotinate. However, it was shown that this was unlikely since ester M, upon treatment with methanolic potassium methoxide at room temperature for three days, did not yield either methyl <u>epi</u>annotinate or annotinine hydrate.

In the proceedure for the isolation of ester M the absolute methanolic reaction mixture was evaporated to dryness and water added to the residue which partially dissolved to leave the water insoluble ester M.

The assumption that water was not involved in the formation of ester M was largely verified by the infrared spectrum (nujol) of the residue obtained by evaporation of the absolute methanol from the reaction solution, since it exhibited bands at 3430 and 1724 $cm.^{-1}$ which are of the same frequency as the equivalent bands that are shown by ester M.

Cole (23) in 1954 reported that steroidal cyclopropane rings exhibit a peak in the infrared spectrum at approximately 3050 $cm.^{-1}$. It was therefore hoped that a high resolution infrared spectrum of ester M would show the presence of a band at approximately 3050 $cm.^{-1}$. Before the high resolution spectrum was obtained, the low resolution spectra (fluorohalogen oil) of ester M and methyl <u>epi</u>annotinate were compared. Methyl <u>epi</u>annotinate exhibited a sharp band at 3030 $cm.^{-1}$ while ester M exhibited a broad band between 2500 and 3300 $cm.^{-1}$. Since methyl <u>epi</u>annotinate XXX(charts IV, V and VII, pages 24, 28, and 40) does not contain a cyclopropane ring, the reliability of this proceedure seemed very doubtful. This view was confirmed by Allen, Davis, Humphlett and Stewart (27) who have reported the existence of many compounds that contain a cyclopropane ring but which do not exhibit a peak at 3050 $cm.^{-1}$ in the infrared spectrum.

A nuclear magnetic resonance spectrum could not be obtained since neither ester M nor any of its derivatives was soluble in suitable solvent.

Structure IL for ester M contains a 1:2 diol system and a negative qualitative test involving periodic acid indicated, but did not prove, that it was not present and hence a more vigorous proof was performed.

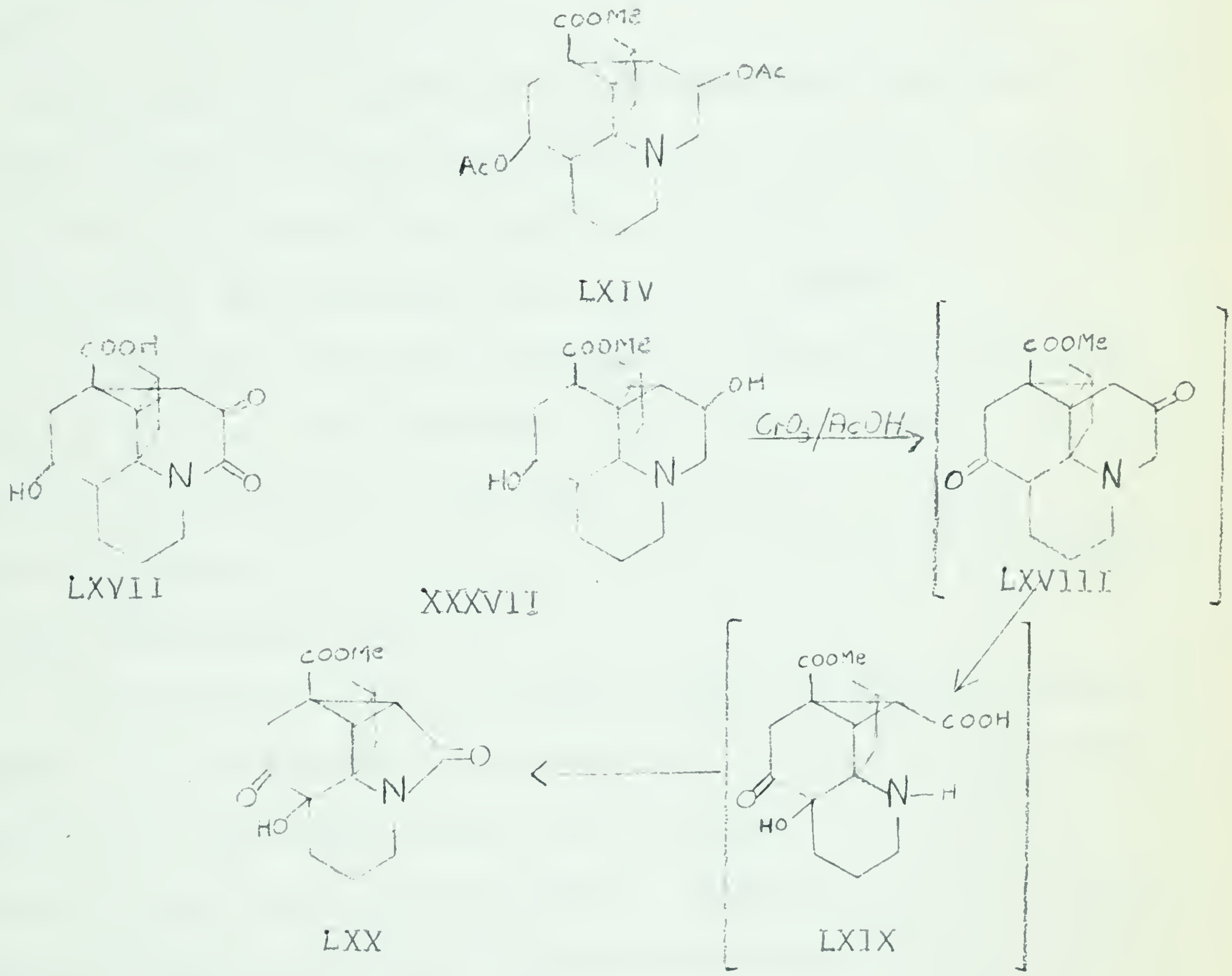
A methanolic solution of ester M and periodic acid was refluxed for 90 minutes and worked up in the usual manner to yield unchanged ester M (90% recovery).

The time curve for the reaction of periodic acid with ester M was plotted. It was found that after 4 days the blank had taken up as much periodic acid as ester M.

The fact that periodic acid failed to react with ester M did not rule out IL as a possible structure for ester M, since if there was a trans diaxial relationship between the two hydroxyls then the periodic acid would be expected to oxidise ester M infinitely slowly. The action of lead tetraacetate, which will react with trans diaxial 1:2 diols was, therefore, investigated.

Standard solutions of ester M, annotinine hydrate and annotininediol and also a blank were treated with equimolecular proportions of lead tetraacetate. After 84 hours the same small amount of lead tetraacetate had been reduced in each of the solutions and the addition of water to each of the solutions did not make any difference to the rate of reduction

CHART VIII



LXIV

LXVII

XXXVII

LXVIII

LXIX

LXX

of the lead tetraacetate. The experiment was repeated, using ester M hydrochloride, annotininediol hydrochloride, mannitol and a blank. Again there were no significant differences in the rates of consumption of the lead tetraacetate by the blank and ester M but the mannitol and the annotininediol hydrochloride solutions consumed the lead tetraacetate fairly rapidly, as expected.

The failure of periodic acid and lead tetraacetate to react with ester M strongly indicated that a 1:2 diol system was not present in ester M and hence that ester M does not possess the structure IL but rather the structure XXXVII.

The chromic acid acid oxidation of ester M was the next reaction that was investigated. A solution of ester M in a solution of pyridine and chromium trioxide was stirred at room temperature for 4 days and worked up in the usual manner to yield unchanged ester M.

The ester M was then dissolved in a solution of aqueous acetic acid and chromium trioxide and the resulting solution was kept at room temperature for 48 hours. The reaction solution was worked up in the usual manner to yield unchanged ester M (91% recovery). A large excess of chromium trioxide was then added to a solution of ester M in glacial acetic acid at 100°. Under these vigorous conditions a neutral, white solid was obtained, which analysed as $C_{16}H_{19}O_5N$. Initially it was thought that the product was a hydroxyketo-amidocarboxylic acid LXVII(chart VIII, page 44) since bands were exhibited in the infrared spectrum (nujol) at 3280 cm^{-1}

(OH), 1726 $cm.^{-1}$ (-COOH), 1696 $cm.^{-1}$ (ketone) and 1673 $cm.^{-1}$ (ring A, 6 membered lactam). The main objection to this structure was that the spectrum did not show any absorption in the region around 2600 $cm.^{-1}$ which is characteristic of the O-H stretching of a carboxylic acid. An attempt was therefore made to esterify the acid by treating a methanolic solution with an ethereal solution of diazomethane. The infrared spectrum (nujol) of the product was identical to that of the starting material which indicated that a carboxyl group was not present.

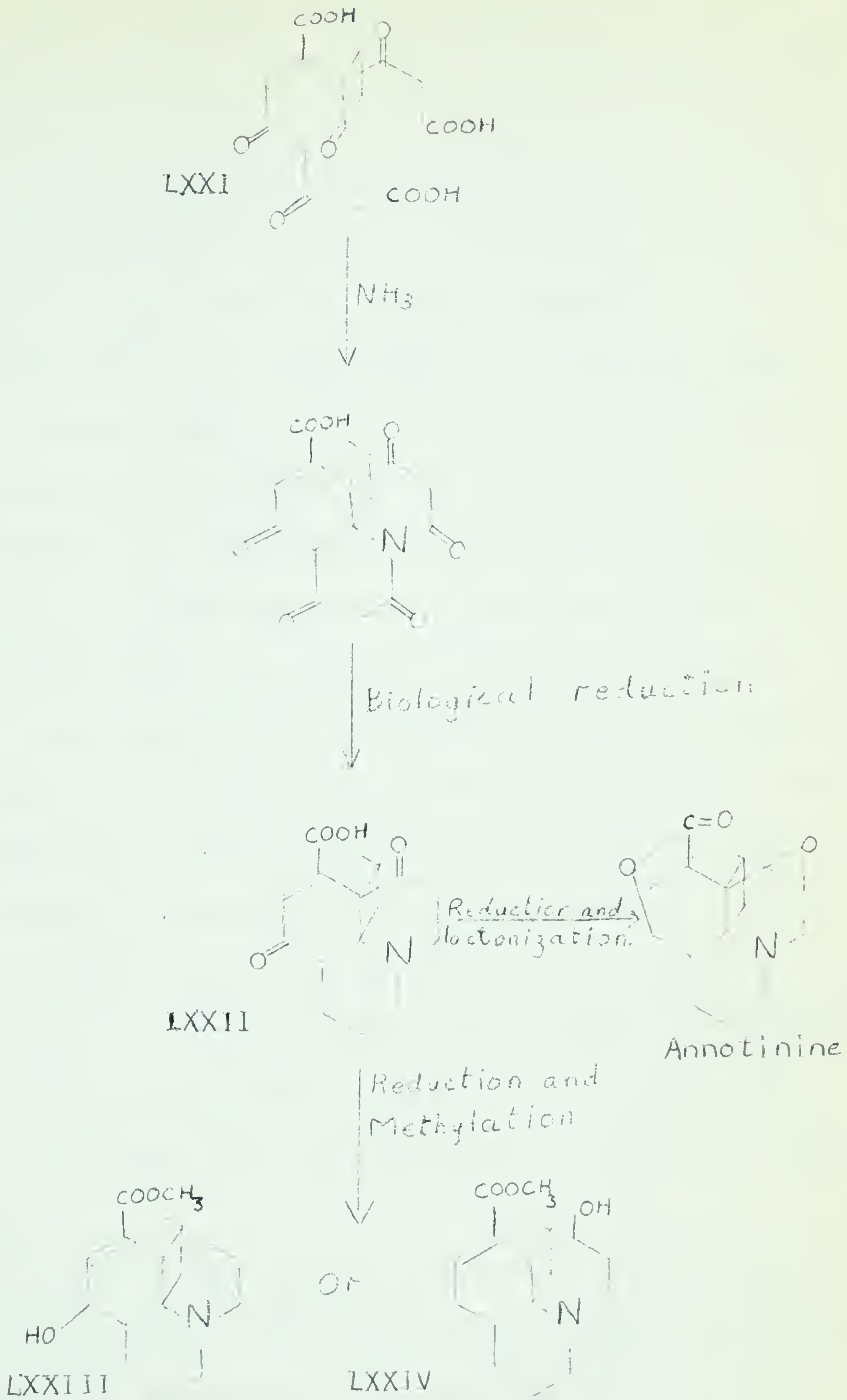
The formation of the $C_{16}H_{19}O_5N$ compound on vigorous chromic acid oxidation is consistent with structure XXXVII, but not IL, for the ester M. Thus oxidation of XXXVII to the amino acid LXIX (chart VIII, page 44) perhaps via the diketone LXVIII (chart VIII, page 44) and lactamization would give LXX (chart VIII, page 44) which would be relatively stable to further oxidation. The oxidation of the ring B hydroxyl, via the ketone, to the formulated α-hydroxyketone has been observed in several derivatives of annotinine. Structure IL, however, does not explain the incorporation of a tertiary hydroxyl group. It should be mentioned at this point that the low infrared frequency (below 1725 $cm.^{-1}$) at which the carbomethoxy group absorbs in ester M and its derivatives is consistent with its attachment to a cyclopropane ring (25).

Structure XXXVII, which appears to be consistent with all the known chemistry of ester M is therefore advanced as the structure of this interesting substance. Inspection of models indicated that the compound is not impossibly strained.

PREPARATION OF METHYL DESOXIDOANNOTINATE.

A hitherto unreported alkaloid was isolated in 1959 in

CHART IX


COOH
COOH
COOH
LXXI
NH_3
COOH
N
Biological reduction
COOH
N
LXXII
Reduction and lactonization
C=O
N
Annotinine
Reduction and Methylation
$COOCH_3$
HO
N
LXXIII
or
$COOCH_3$
OH
N
LXXIV

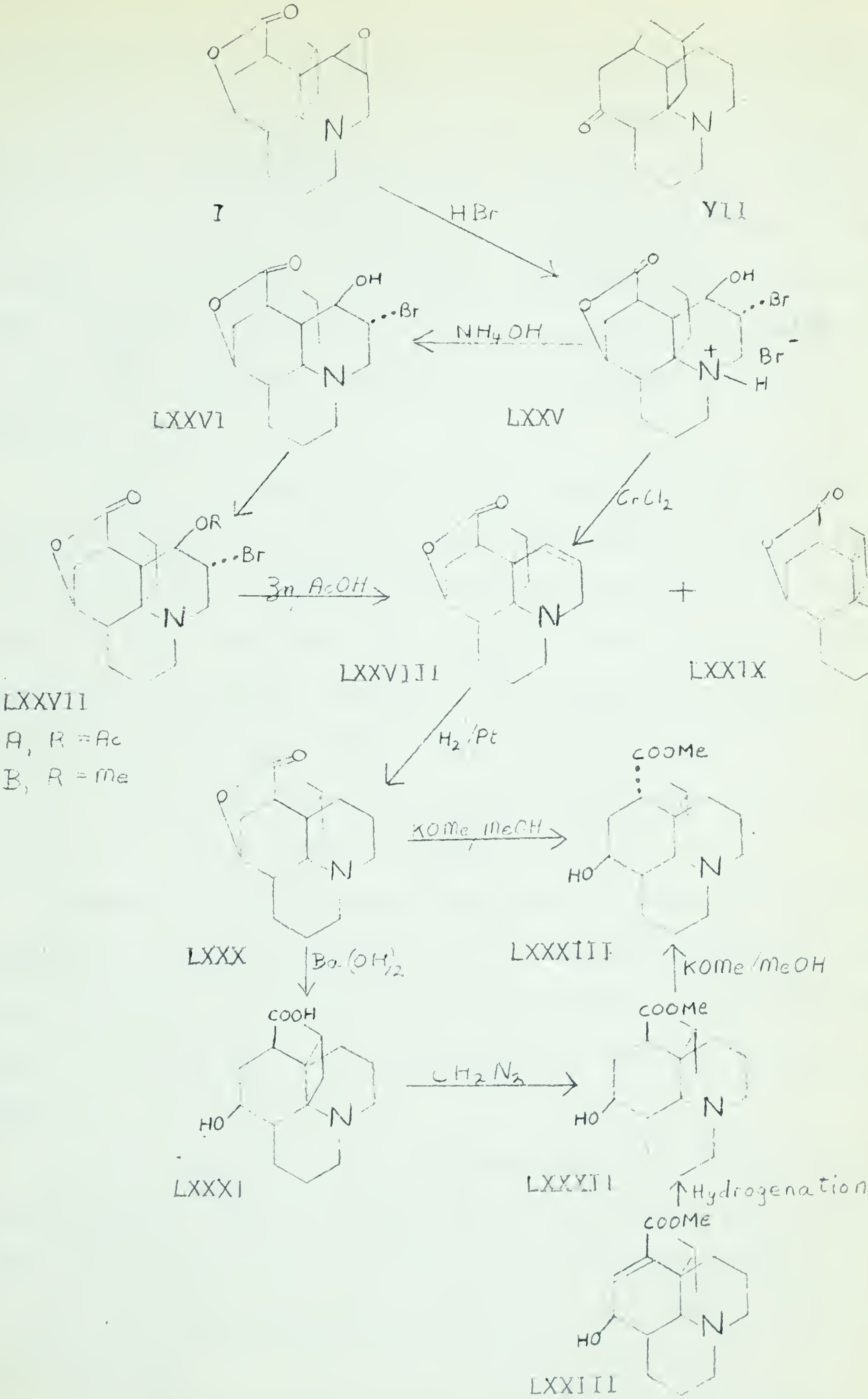
this department (26) from Lycopodium annotinum L. It has been tentatively called "olivine" and was shown (26) to have the molecular formula, $C_{17}H_{25}O_3N$. Olivine is a hydroxy methyl ester(26) and the infrared and ultraviolet spectra indicated that it was an $\alpha:\beta$-unsaturated ester. It does not contain an N-methyl group but does have one C-methyl group.

Approximately 400 mg. of "olivine" were isolated from 145 lb. of dry plant, which rules out the possibility of a rigorous degradative elucidation of its structure.

Biogenetically, it is considered (27) that annotinine is derived from an intermediate of the type LXXI (chart IX, page 47) via LXXII (chart IX, page 47). The intermediate LXXII leads to two reasonable structures, LXXIII and LXXIV (chart IX, page 47) for "olivine". Structure LXXIII has now been eliminated as a possibility by the synthesis of the two possible dihydro derivatives LXXXII and LXXXIII (chart X, page 49) of LXXIII (chart IX, page 47). The synthesis of LXXXII and LXXXIII is now described. Both LXXXII and LXXXIII could theoretically be prepared by one of the routes shown diagrammatically on chart X. Annotinine was used as the starting material.

The first step, which was the preparation of annotinine bromohydrin hydrobromide LXXV (chart X) was easily performed. Although annotinine bromohydrin hydrobromide was used in the X-ray analysis of annotinine (41), details of its preparation are not given in the literature. It was found that if annotinine was dissolved in the minimum of aqueous hydrobromic acid (48%)

CHART X


I
VII
HBr
LXXVI
NH_4OH
LXXV
$CrCl_2$
LXXVII
A, R = Ac
B, R = Me
Zn AcOH
LXXVIII
+
LXXIX
H_2/Pt
LXXX
KOMe, MeOH
COOMe
LXXXIII
$Ba(OH)_2$
COOH
LXXXI
CH_2N_2
LXXXII
KOMe/MeOH
Hydrogenation
LXXIII

for solution and then refluxed for a few minutes, cubic crystals of annotinine bromohydrin hydrobromide precipitated in almost quantitative yield (98.7% yield). The salt was not analysed since its spectral properties could only be consistent with bromohydrin formation. The free base, annotinine bromohydrin LXXVI (chart X, page 49) was readily prepared. The salt was treated with ammonium hydroxide and chloroform to yield the free base.

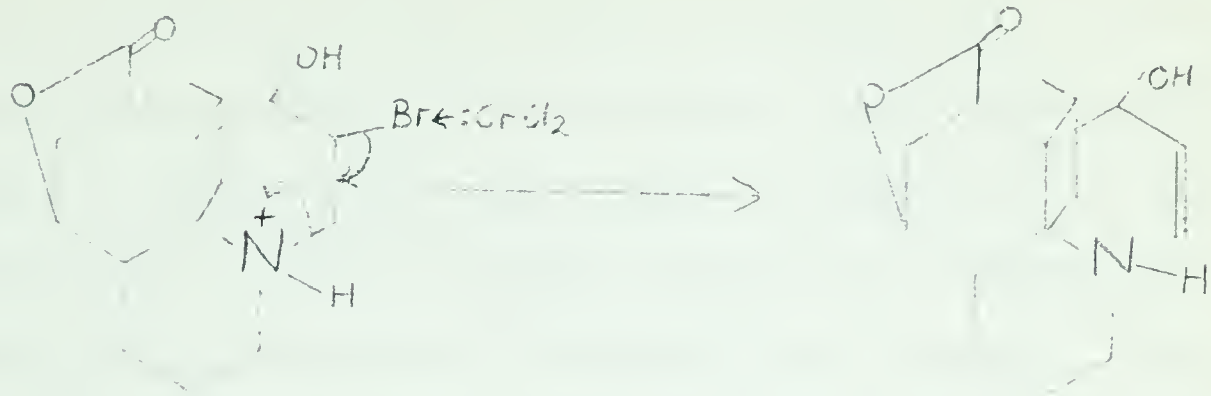
The next compound in the series, LXXVIII, had already been prepared by Marion and co-workers (28) who had treated annotinine chlorohydrin hydrochloride with chromous chloride, but his yield was less than 30%. It was hoped that either acetoxyannotinine bromohydrin LXXVIIA (chart X, R=Ac) or methyl annotinine bromohydrin LXXVIIB (chart X, R=Me) could be prepared in high yield, and converted also in high yield, by the action of zinc and acetic acid to LXXVIII.

Treatment of annotinine bromohydrin hydrobromide with acetic anhydride in pyridine yielded, after chromatography, a brown oil whose infrared spectrum (chloroform) was in accord with that expected for acetoxyannotinine bromohydrin LXXVIIA (chart X, R=Ac, page 49)

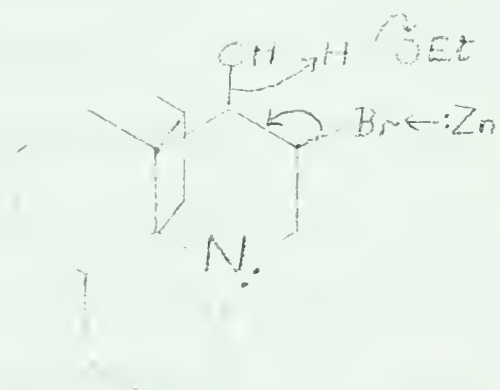
In an attempt to form the methyl ether of annotinine bromohydrin LXXVIIB (chart X, R=Me, page 49), annotinine bromohydrin LXXVI was treated with a solution of fluoroboric acid in methylene chloride and diazomethane in ether. Annotinine bromohydrin was recovered.

Since neither LXXVIIA nor LXXVII B had been prepared in a

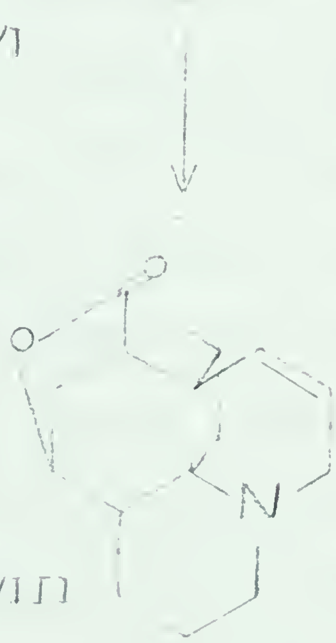
CHART XI



LXXV LXXIX



LXXVI



LXXVIII

crystalline state, annotinine bromohydrin was treated directly with zinc and acetic acid and later with zinc and ethanol, since Fieser and Ettore (29) had removed the hydroxyl and bromine groups from various bromohydrins, by these proceedures. With zinc and acetic acid, starting material was recovered and the O-acetate of the starting material was the only other product isolated. With zinc and ethanol, elimination of the bromine occurred to yield a hydroxy unsaturated lactone that had previously been obtained by Marion and co-workers (28), upon treating annotinine chlorohydrin hydrochloride with chromous chloride and hydrochloric acid. Under acid conditions, the mechanism for the production of the hydroxy unsaturated lactone LXXIX (chart X, page 49) involves the withdrawal of C-N bonded electrons to the electron deficient N^{+} atom, as shown on chart XI (page 51). It was hoped that under neutral conditions LXXIX would not be obtained in any quantity since the nitrogen would no longer be as electron deficient and LXXVIII (chart X, page 49) would be preferentially obtained by the mechanism shown on chart XI. But evidently the tertiary nitrogen is still the best leaving group.

In the final attempt to prepare LXXVIII directly from annotinine bromohydrin, annotinine bromohydrin was treated with a large excess of zinc in acetic anhydride (which can be refluxed at a higher temperature than acetic acid or ethanol). The only product was the O,N-diacetyl derivative of the hydroxy unsaturated lactone LXXIX (chart X, page 49).

Only starting material was recovered when the oily acetoxyannotinine bromohydrin was treated with zinc and ethanol.

Since all the sttempts to prepare LXXVIII (chart X, page 49), under neutral conditions had failed, it was prepared by the method of Marion and coworkers (28), using annotinine bromohydrin, chromous chloride and hydrochloric acid.

Compound LXXVIII (dehydrodesoxidoannotinine) was hydrogenated to yield desoxidoannotinine LXXX (chart X, page49) which on treatment with aqueous barium hydroxide yielded desoxidoannotinic acid LXXXI (chart X, page49) as a semi-solid. The amino acid LXXXI was characterised as its methyl ester which was prepared by treating the acid with diazomethane.

Finally the epimer of methyl desoxidoannotinate, methyl _epi_desoxidoannotinate LXXXIII (chart X, page49) was prepared from both methyl desoxidoannotinate and desoxidoannotinine by the action of absolute methanolic potassium methoxide. The preparation from desoxidoannotinine required a very large excess of methoxide and a long reaction time.

Unfortunately, "olivine" did not yield either methyl _epi_desoxidoannotinate or methyl desoxidoannotinate upon hydrogenation and hence "olivine" does not possess the structure LXXIII.

Although neither methyl desoxidoannotinate nor its epimer were identical to "dihydroolivine", there is a possibility that in the future they may be of use in determining the structures of the three other C_{17} alkaloids that have been isolated from various species of the _Lycopodium_ family. The alkaloids are, L 28, $C_{17}H_{27}O_2N$, which was isolated by Manske and Marion (30)

from Lycopodium acrifolium (fern); clavatoxine, $C_{17}H_{27}O_2N$, which was isolated by Achmatowicz (31) from Lycopodium clavatum L. and L20, $C_{17}H_{27}O_2N$, which was isolated by Manske and Marion (32) from Lycopodium lucidulum Michx. These three alkaloids all contain 17 carbon atoms and 2 oxygen atoms and hence from the biogenetic scheme which has recently been outlined by Conroy (27), if they do not contain an N-methyl group, the seventeenth carbon atom must be present as a methyl ester. There is therefore a reasonable possibility that if either methyl desoxidoannotinate or methyl epidesoxidoannotinate can be dehydroxylated, then one or possibly two of the C_{17} alkaloids will be obtained.

EXPERIMENTAL

All melting points have been corrected unless otherwise stated. The melting points were obtained on a Fisher-Johns Melting Point Apparatus, using Corning Cover Glasses.

The infrared spectra were recorded on a Perkin -Elmer Recording Infrared Spectrometer, Model P.E. 21, using sodium chloride sealed cells.

The ultraviolet spectra were recorded on a Cary Recording Spectrophotometer, Model 14M, using quartz 1 cm. cells.

The proceedure adopted for all the chromatographs was to dissolve the material that was to be chromatographed in the solvent with which it was to be put on the column and then evaporate to dryness, redissolve, evaporate, redissolve and evaporate. The compound was then dissolved in the solvent and placed on the column. Only purified solvents were used. Standard chromatographical proceedures were then used.

EXTRACTION OF LYCOPODINE, XVI FROM LYCOPODIUM CLAVATUM L. The method used was that of Manske and Marion (9). Lycopodium Clavatum L. (16.1 kg.) was air dried, placed in percolators and methanol percolated through until the percolates were colourless. This required approximately 15 to 20 litres per kilogram. Most of the methanol was removed from the extracts by distillation and then completely removed azeotropically with water. Dilute hydrochloric acid (6 N) was then added to the resulting viscous, green mass until the resulting dark green solution was acid to Congo Red. Water (1 l. for every 2 kg. of plant) was added to the acidic solution, which was then stirred overnight at 70°, cooled and put aside for one day.

The dark brown, aqueous solution, was then decanted from the black glue-like mass of fats, which was then heated twice more with very dilute hydrochloric acid and the decanted material obtained was added to the first decantate. In order to obtain a clear solution the aqueous extract was filtered through charcoal and the majority of the neutral and acidic compounds present in aqueous solution removed by repeated chloroform extraction, until the chloroform was colourless.

The aqueous, acidic extract was basified with ammonia and extracted four times with equal quantities of chloroform. The chloroform extract was then filtered through charcoal, dried over anhydrous sodium sulphate and the chloroform removed to yield a brown viscous mixture of crude alkaloids (18.075 g.).

A solution of the crude alkaloids in benzene was chromatographed on basic alumina (460 g.). Elution with benzene (2,100 ml.) yielded crude lycopodine (6.574 g.) m.p. 114° (lit. 116°) I.R. ($CHCl_3$), 1701 cm.$^{-1}$.

PURIFICATION OF CRUDE LYCOPODINE, XVI. The crude lycopodine obtained from the chromatogram was dissolved in ether and the solution concentrated to give colourless, prismatic crystals (2.522 g.) m.p. 115.5° (lit. 116°). The mother liquors were dissolved in acetone and a 17% aqueous solution of perchloric acid added until the pH. was 7. Cream coloured, poorly formed crystals of the perchlorate immediately separated. Recrystallization from methanol yielded colourless prisms (4.338 g.) m.p. 282° (lit. 282°).

Lycopodine perchlorate was converted to the free base (3.128 g.) m.p. 115° (lit. 116°), by dissolving the salt in dilute ammonia and extracting the free base with chloroform.

The infrared spectrum of lycopodine in chloroform showed strong carbonyl absorption at 1701 cm.$^{-1}$.

LYCOPODINE HYDROBROMIDE.

A. Lycopodine (224 mg.) was dissolved in the minimum quantity of glacial acetic acid (3 ml.) necessary for solution and to this was added a solution of hydrogen bromide (11 mg.) in glacial acetic acid (1.2 ml). Bromine (156 mg., 0.9 equivalent) in glacial acetic acid (4.2 ml.) was added to the colourless solution of lycopodine. Immediately a pale yellow precipitate (257 mg.) was obtained.

Crystallisation of the yellow solid from a mixture of methanol and acetone gave white prisms (191 mg., 64% yield). The compound did not melt below 315°. The infrared spectrum, ($CHCl_3$) exhibited peaks at 2490 and 1718 cm.$^{-1}$.

Attempts to isolate further pure products were unsuccessful.

B. Lycopodine (19 mg.) was dissolved in the minimum quantity of anhydrous ether necessary for solution and anhydrous hydrogen bromide passed into the colourless solution until the solution was saturated. After two or three minutes at room temperature, poorly formed, pale yellow crystals (25 mg., 99% yield) precipitated from solution.

The infrared spectrum in chloroform was very poorly resolved, but on washing the yellow crystals with acetone the

yellow colour was removed and the infrared spectrum much improved. Crystallisation from acetone-methanol gave colourless prisms which showed bands in the infrared ($CHCl_3$) at 2480 and 1720 $cm.^{-1}$. (For spectrum, see I.R. -1). The crystals did not melt or decompose below 315°.

MONOBROMOLYCOPODINE HYDROBROMIDE. Lycopodine hydrobromide (590 mg.) was dissolved in the minimum quantity of chloroform necessary for solution. Bromine (288 mg., I.O. equivalent) dissolved in a small volume of glacial acetic acid was added to the lycopodine hydrobromide solution. Decolorisation took place immediately.

The solution was concentrated to one third of the original volume. Pale yellow microcrystals (543 mg., 74% yield) of monobromolycopodine hydrobromide separated.

An analytically pure sample was obtained as colourless needles, after repeated recrystallisations from an acetone-methanol mixture. The crystals decomposed in the range of 250° to 259° and the infrared spectrum ($CHCl_3$) (spectrum I.R. -2) showed peaks at 2455 $cm.^{-1}$, which is characteristic of a quaternary ammonium salt and at 1730 $cm.^{-1}$, which is characteristic of a six membered carbocyclic ketone. Monobromolycopodine hydrobromide analysed as $C_{16}H_{25}ONBr_2$. Calculated: C-47.19%, H - 6.19%, O - 3.93%, N - 3.46%, Br - 39.23%. Found C - 47.65%, H - 6.26%, O - 3.4%, N - 3.5%, Br - 39.19%.

ATTEMPTED DI OR TRIBROMINATION OF LYCOPODINE. A chloroform solution of lycopodine hydrobromide (32 mg.) was treated with

bromine (37.5 mg., 2.4 equivalents) dissolved in a small quantity of glacial acetic acid. Most of the bromine (2.2 equivalents) was decolourised by this proceedure. The remaining 0.2 equivalent was in excess.

The volume of the solution was reduced to one third of its original volume and the solution cooled. Pale yellow needles (14 mg., 43% gram yield) were obtained, which did not melt below 315°. Recrystallisation of the yellow needles from a mixture of methanol and acetone yielded colourless needles whose infrared spectrum ($CHCl_3$) was identical to that of monobromolycopodine hydrobromide ($CHCl_3$). There was insufficient material for an analytically pure sample to be prepared.

All the solvent was removed from the mother liquids to yield a brown oil. Crystalline material was not obtained from the brown oil.

HYDROLYSIS OF MONOBROMOLYCOPODINE. The method used was that of Barclay and MacLean (5). Monobromolycopodine hydrobromide (70 mg.) was suspended in freshly purified dioxane. Aqueous 5% potassium hydroxide (5 ml.) was added until a homogeneous solution was obtained. The colourless starting solution rapidly turned pale yellow. After stirring the solution for one minute at 0°, it turned a yellow colour and then gradually darkened until it was light brown in colour, after three minutes. It was left for a further thirty minutes at 0°. The colour did not intensify any further.

All solvent was removed _in vacuo_ at room temperature to

yield a brown oil. Ice-cold water was added to the brown oil and the resulting brown, aqueous, basic solution was worked up in the usual manner. Although the ultraviolet and infrared spectra indicated the introduction of unsaturation into the molecule, the amorphous products resisted attempts at purification.

ATTEMPTED PREPARATION OF DIKETO LYCOPODINE, IX, FROM BROMO-LYCOPODINE. In this experiment all the mother liquors of the previous bromination experiments on lycopodine were used. They were all mixed and the solvent removed at 60° under reduced pressure. The dark brown residue obtained was dissolved in chloroform and the resulting dark brown solution was filtered. An excess of liquid bromine (500 mg.) in glacial acetic acid was added to the filtrate. The solution was left standing overnight at room temperature and then all the solvent was removed at 60° under reduced pressure. A black oil was obtained which was then dissolved in 5% aqueous potassium hydroxide, containing a little dioxane. Further aqueous potassium hydroxide was added, until the solution was pH 9. The black solution was heated on the steam bath for 30 minutes, then cooled and solid carbon dioxide added until the solution was pH 7.

The neutral, aqueous solution was continuously extracted with ether for three days. The ethereal extract obtained was washed with water and then dried over anhydrous magnesium sulphate. The ether was evaporated at 30°, under reduced pressure

and the resulting product was a brown, oily solid (378 mg.) I.R. ($CHCl_3$) 3390 cm.$^{-1}$, (OH bonded), 1703, 1688 and 1632 cm.$^{-1}$.

The compound was dissolved in a chloroform-methanol (100:1, by volume) mixture and filtered through a column of acid washed alumina (5 g.). The colour intensity was not reduced and all the compound was recovered as a viscous oil.

A benzene solution of the viscous oil was chromatographed on "acid-washed" alumina (12 g.). Three main fractions were eluted from the column.

Fraction i, a dark brown semi-solid (60 mg.) was obtained with chloroform elution, I.R. ($CHCl_3$) 3400, 1720, 1632 and 1605 cm.$^{-1}$, U.V. (95% EtoH)λmax. 315 mμ(E, 9.500). Absorption maxima were not exhibited in the visible region of the spectrum.

Fraction ii, a reddish oil (251 mg.) was obtained with chloroform and methanol (19:1) elution, I.R. ($CHCl_3$) 3450, 1722, 1665 and 1633 cm.$^{-1}$, U.V.(95% EtoH)λ max. 257 mμ ($\mathcal{E}$, 5,600) and 332 ($\mathcal{E}$,4,300) Absorption maxima were not observed in the visible region of of the spectrum and attempts to prepare a crystalline 2,4 - dinitrophenylhydrazone were unsuccessful.

Fraction iii, a brown oil (61 mg.) was obtained with methanol elution, I.R.($CHCl_3$) 3450, 1720, 1665 and 1633 cm.$^{-1}$, U.V. (95% EtoH)λ max 255 mμ($\mathcal{E}$, 3,300). Absorption maxima were not observed in the visible region of the spectrum. The compound could not be induced to crystallise, nor could crystalline derivatives be obtained.

ATTEMPTED PREPARATION OF DIKETOLYCOPODINE IX FROM MONOBROMO-LYCOPODINE HYDROBROMIDE. The method used was that of Kornblum (14). A solution of monobromolycopodine hydrobromide (30 mg.) in dimethyl sulphoxide was left standing under anhydrous conditions for seven weeks at room temperature. The

chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and the solvent swirled off at 60° under reduced pressure.

A yellow semi - solid (16 mg.) which would correspond to an 84% yield of the diketone was obtained. The infrared spectrum ($CHCl_3$) possessed bands at 3720, 3450, 2450, 1740, 1705 and 1640 $cm.^{-1}$. The first two bands could be given by an enolic hydroxyl group and the 1640 $cm.^{-1}$ peak by an enolic double bond. The 2450 $cm.^{-1}$ band indicates the presence of a quaternary ammonium salt, and the 1705 $cm.^{-1}$ band indicates that the normal keto group of lycopodine could now be conjugated. The 1740 $cm.^{-1}$ peak appears to be anomalous.

The semi - solid resisted all attempts to crystallise it and did not sublime, but distilled above 200° at 0.02 mm. pressure as a yellow oil. Neutral alumina was not available at the time for chromatography.

A further experiment was not performed because further lycopodine was not available at that time.

ACTION OF SELENIUM DIOXIDE ON LYCOPODINE. A solution of lycopodine (110 mg., 1 equivalent) and selenium dioxide (120 mg., 3 equivalents) in dioxane (15 ml.) was refluxed for 4 hours.

The red reaction solution was filtered and the filtrate was evaporated to dryness at 60°, _in vacuo_, to yield a red oil. An aqueous solution of the red oil was basified with concentrated ammonium hydroxide and was then methylene chloride extracted. The extract was washed with water, dried over

anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a red oil.

A solution of the oil in benzene was chromatographed on neutral, "Woelm" alumina (1 g., Grade I). Elution with benzene yielded lycopodine (73 mg., 65% recovery), while elution with benzene-ether (3:1) yielded a colourless viscous oil (24 mg.), whose infrared spectrum ($CHCl_3$) was identical to that of lycopodine. Elution with chloroform-methanol (99:1) yielded a red oil (11 mg.) I.R. ($CHCl_3$) 1707, 1607 and 1643 $cm.^{-1}$. The ultraviolet spectrum (95% EtoH) did not exhibit any maximal absorption.

The overall recovery of lycopodine was 97 mg. (89%).

ZIMMERMANN TEST ON LYCOPODINE, XVI. The method used was that of Zimmermann (16). The test was performed on three samples. The first was a concentrated ethanolic solution of lycopodine, the second was a blank of ethanol, and the third was acetone.

Ethanolic 1% meta dinitrobenzene (0.05 ml.) and aqueous potassium hydroxide (1 ml.) were added to 0.05 ml. of each of the three solutions. The acetone took one day, the ethanol two days, and the lycopodine solution four days to develop the same pale yellow colour. On the fourth day the blank and the acetone had changed to a fairly dark orange-brown colour.

EXTRACTION OF ANNOTININE, I. The proceedure used was that of Manske and Marion (9).

The source of annotinine that was used was dry <u>Lycopodium</u>

annotinum L. (65 kg.). The same proceedure as that used for Lycopodium clavatum L. was employed to obtain a solution containing only crude alkaloids in chloroform. Instead of removing all the chloroform from the pale yellow solution the volume was reduced to 500 ml. An equal volume of 98% ethanol was added to the dark brown viscous solution. The volume was again reduced to 500 ml. and a further 500 ml. of ethanol added. This proceedure was repeated until all the chloroform had been replaced, at which point annotinine crystallised out of the hot ethanolic solution. The solution was cooled, the annotinine filtered off and washed twice with ice-cold 98% ethanol. Almost pure annotinine (73 g.) was obtained as white needles, m.p. 231° (lit. 232°). The infrared spectrum ($CHCl_3$) was identical to that of an authentic sample of annotinine.

All the ethanol was removed from the mother liquor. The dark brown oil obtained was dissolved in chloroform and the chloroform solution was shaken with dilute hydrochloric acid. Concentrated ammonia was then used to basify the aqueous, acidic solution. Chloroform extraction of the aqueous, basic solution gave a light brown solution, which was then dried over anhydrous sodium sulphate and evaporated to dryness at 60° under reduced pressure. A black residue was obtained which was dissolved in 95% ethanol. The ethanolic solution was reduced to half its original volume, cooled and seeded to yield annotinine (3g.).

My supervisor, Dr. W.A. Ayer, and a colleague, G.G. Iverach, later isolated a further yield of annotinine (14 g.)

Thus a total of 90 g. was isolated which represented a yield of almost 1.4 g. per kg. of plant, while the literature usually quotes a value of 0.5 g. per kg. of plant.

ANNOTININE LACTAM, II. Annotinine (10 g. 36.1 millimoles) was dissolved in a solution of oxalic acid (3.75 g., 30 millimoles) in water (100 ml.). From a standard solution of potassium permanganate (30 g.) in water (4 l.), potassium permanganate (10.1 g., 68 millimoles) was added dropwise, with stirring at room temperature, to the solution of annotinine. This quantity was theoretically sufficient to oxidise both the annotinine and the oxalic acid. More of the potassium permanganate solution (600 ml.) was then added until a permanent pink colour was obtained that lasted for about three minutes.

Sulphur dioxide was passed into the reaction mixture until all the precipitated manganese dioxide had been reduced to water soluble manganous ions. A flocculent, white precipitate of annotinine lactam was then present in an otherwise clear solution. The annotinine lactam (2 g.) was obtained by filtration, m.p. 234° (lit. 234°). The aqueous filtrate was chloroform extracted. The extract was dried over anhydrous sodium sulphate and the chloroform removed at 60° under reduced pressure. By this means a further yield of the lactam (4.4 g.) was obtained which after recrystallisation from hot methanol gave white cubes, m.p. 234°, I.R. ($CHCl_3$) 1683 and 1648 cm.$^{-1}$ (lit. 1775 and 1645 cm.$^{-1}$).

The aqueous solution that had been chloroform extracted

was made strongly acidic (pH 1) with concentrated sulphuric acid and continuously extracted with ether for four days. No more annotinine lactam was obtained.

The overall yield of annotinine lactam was 60%.

ANNOTININE CHLOROHYDRIN LACTAM, III. The method used was that of MacLean and coworkers (33).

A solution of annotinine lactam (21.3 g.) in concentrated hydrochloric acid was refluxed for 40 minutes and then cooled. Grey prisms of annotinine lactam (17 g.) crystallised from the reaction solution and were removed by filtration. The filtrate was then chloroform extracted. The extract was washed with dilute, aqueous sodium bicarbonate, dried over anhydrous sodium sulphate and evaporated to dryness at 60° under reduced pressure. A further yield of the chlorohydrin (3.4 g.) was obtained.

The mother liquors of the crystallisation were evaporated to dryness. The residue was dissolved in concentrated hydrochloric acid and the solution refluxed for one hour. The clear solution was cooled and chloroform extracted. The extract was washed with with dilute, aqueous sodium bicarbonate and then water. The extract was evaporated to dryness at 60°, under reduced pressure. The residue consisted of annotinine chlorohydrin lactam. (0.5g.)

All the various fractions of annotinine chlorohydrin lactam were combined and recrystallised from methanol. A colourless conglomerate (19.5 g., 89% yield) was obtained, m.p. 296° (lit. 297°), I.R. ($CHCl_3$) 3400, 1780, and 1630 $cm.^{-1}$.

ANHYDROANNOTININE CHLOROHYDRIN LACTAM, IV. The method used was that of MacLean and coworkers (33).

A solution of annotinine chlorohydrin lactam (19.5 g.) in phosphorus oxychloride was refluxed for 90 minutes. The phosphorus oxychloride was removed from the dark brown solution obtained, at 45°, under reduced pressure. The residue obtained was a brown oil. Water was then added to the oil, to decompose any phosphorus oxychloride that was still present. A two phase liquid system was obtained that consisted of a brown aqueous solution and a brown oil. The two phase system was chloroform extracted. The chloroform extract was washed with dilute, aqueous sodium bicarbonate, then water and finally dried over anhydrous sodium sulphate. Evaporation of the chloroform solution, at 30°, under reduced pressure yielded a brown oil. The oil was crystallised from methanol and white rhomboids (8.0 g.) were obtained, m.p. 184° (lit. 187°).

The mother liquor was evaporated to dryness to yield a brown solid residue. A solution of the residue in chloroform was chromatographed on " acid washed " alumina (200 g.). Elution with chloroform yielded light brown coloured anhydroannotinine chlorohydrin lactam (14.943 g.).

Recrystallisation of the original 8 g. and the 14.943 g. from methanol, yielded colourless rhomboids of the anhydro compound (14.1 g. 76% yield), I.R. ($CHCl_3$) 1787 $cm.^{-1}$ (γ-lactone), 1665 $cm.^{-1}$ (double bond in conjugation with the lactam) and 1618 $cm.^{-1}$ (α-β- unsaturated lactam) (lit. I.R. ($CHCl_3$) 1782, 1662, and 1616 $cm.^{-1}$).

DESOXIDOANNOTININE LACTAM, V. The method of MacLean (33) was used.

A basic (NH_4OH, 2ml.), methanolic solution of anhydro-annotinine chlorohydrin lactam (14 g.) was added to a magnetically stirred suspension of pre - reduced, finally divided Adams catalyst (2.325 g.) in methanol. Purified hydrogen was then passed over the mixture at atmospheric pressure and at room temperature. After 3 days no further hydrogen was absorbed.

White crystals were present in the mixture. Ethanol was added to dissolve the crystals. The black platinum was then filtered off and the colourless solution evaporated to dryness <u>in vacuo</u> at room temperature to yield a white residue. The residue was dissolved in a mixture of chloroform and water and the mixture shaken. The chloroform solution was dried over anhydrous sodium sulphate and evaporated to dryness at room temperature under reduced pressure to yield cream coloured rhomboids (9.2 g.). Recrystallisation of the rhomboids from acetone yielded white rhomboids of desoxidoannotinine lactam (8.9 g., 70% yield) m.p. 182° (lit. 181° - 183°) I.R. ($CHCl_3$) 1780 $cm.^{-1}$ (δ lactone) and 1615 $cm.^{-1}$ (δ lactam).

METHYL EPIDESOXIDOANNOTINATE LACTAM, VI.

A. The method used was that of Wiesner and coworkers (34). A solution of potassium hydroxide (5.1 g.) in the minimum quantity of methanol necessary for solution was added to a solution of desoxidoannotinine lactam (5.6 g.) in the minimum

amount of methanol necessary for solution. The clear solution obtained was refluxed for 3 hours and then evaporated to dryness at 60° under reduced pressure, to yield a pale yellow solid. Water was added to the pale yellow solid to yield a clear solution, which was than chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness at 60° under reduced pressure to yield a white solid. Recrystallisation of the white solid from ethyl acetate yielded white needles of methyl epidesoxidoannotinate lactam (2.55 g., 47% yield) m.p. 202° (lit. 204° and 210°). I.R. (nujol) 3250 cm.$^{-1}$ (OH), 1738 cm.$^{-1}$ (-COOMe) and 1608 cm.$^{-1}$ (δ lactam)(lit. I.R. (nujol) 3220, 1730, and 1600 cm.$^{-1}$).

The basic aqueous solution that had been chloroform extracted was acidified with dilute hydrochloric acid. Chloroform extraction of the aqueous, acidic solution obtained, yielded a colourless chloroform solution. The colourless solution was washed with water, dried over anhydrous sodium sulphate, and then evaporated to dryness to yield white needles of desoxidoannotinic acid lactam, (2.1 g., 40% yield), m.p. 262° (lit. 262°-264°), I.R. (nujol) 3250 cm.$^{-1}$ (OH), 1700 cm.$^{-1}$ (-COOH) and 1656 cm.$^{-1}$ (δ lactam).

B. Desoxidoannotinic acid lactam (2.1 g.) was dissolved in the minimum amount of methanol necessary for solution. This solution was added to a solution of potassium hydroxide (2.3 g.), in the minimum amount of methanol necessary for solution. The ensuing solution was refluxed for 3 hours and then evaporated

to dryness at 60° under reduced pressure to yield a white solid. The solid was dissolved in water and the basic, aqueous solution was chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness at 60° under reduced pressure to yield a white solid. Recrystallisation of the white solid from ethyl acetate yielded white needles of methyl epidesoxidoannotinate lactam (420 mg., 17.5% yield). This sample of methyl epidesoxidoannotinate lactam exhibited the same physical properties as the sample obtained from desoxidoannotinine lactam. A second crop of crystals from ethyl acetate consisted of a white conglomerate of methyl desoxidoannotinate lactam (1.5 g., 62% yield) m.p. 163° (lit. 165°-166°) I.R. (nujol) 3250 cm.$^{-1}$ (OH), 1730 cm.$^{-1}$ (-COOMe) and 1600 cm.$^{-1}$ (δ lactam) (lit. I.R. (nujol) 3430, 1725 and 1607 cm.$^{-1}$).

DESOXIDOANNOTININEDIOL XXI. A solution of methyl epidesoxidoannotinate lactam (200 mg., 1 equivalent) in the minimum of absolute benzene was rapidly added to a well stirred suspension of lithium aluminium hydride (276 mg., 10 equivalents) in absolute ether. The reaction mixture was refluxed, using a condenser that was fitted with a calcium chloride guard tube, for 24 hours.

The work up used was one developed by Micovic and Mihailovic (35) to give a granular precipitate of aluminium hydroxide. If (n g.) of lithium aluminium hydride were used, then water (n ml.) followed by 15% sodium hydroxide

(n ml.) and finally followed by water (n ml.) again are added to the reaction mixture. This work up was employed and the granular precipitate obtained filtered off and washed with methylene chloride (I l.). Evaporation of the combined filtrates at 30° under reduced pressure yielded desoxidoannotininediol as a colourless viscous oil, I.R. ($CHCl_3$) 3620 $cm.^{-1}$ (OH, non bonded), 3450 $cm.^{-1}$ (OH, bonded) and no carbonyl absorption (spectrum I.R. -3). Previous experience had shown that this viscous oil was extremely sensitive to aerobic oxidation, or contact with ether, or carbon tetrachloride. Although the viscous oil could be crystallised from absolute ether, repeated crystallisations yielded increasingly impure crystals and finally intractable brown oils. Hence, the viscous oil was stored under an atmosphere of hydrogen.

The viscous oil was dissolved in acetone and dilute hydrochloric acid (6N, o.25 ml.) added. Reductions in the volume of acetone solution followed by scratchings did not yield any crystals. The solution was therefore evaporated to dryness under reduced pressure and the small amount of water that was present was removed azeotropically with benzene and 98% ethanol to yield a brown oil. The brown oil was scratched vigorously. A yellow solid was obtained, which after 7 recrystallisations from an acetone - methanol (3:2) mixture yielded analytically pure rhomboids of desoxidoannotininediol hydrochloride m.p. 299° - 300°.

The overall yield of the salt was 185 mg. (95% yield). Analysed as $C_{16}H_{28}O_2NCl.H_2O$. Calculated: C, 60.06%; H, 9.45%; O,15.00%; N, 4.40% and Cl, 11.09%. Found: C, 60.11%;H,9.48%; O, 15.34%; N, 4.31% and Cl 11.06%. Calculated for one mole of water, 5.64%; Found 7.71%.

The dried material analysed as $C_{16}H_{28}O_2NCl$, Calculated, C, 63.63%; H, 9.35%; and O, 10.60%. Found, C, 63.25%; H, 9.22%; and O, 10.77%.

MONOTOSYLATE OF DESOXIDOANNOTININEDIOL. Unpurified desoxidoannotininediol (380 mg., 1.0 equivalent) in the form of pale yellow, poorly formed crystals was dissolved in a minimum of absolute pyridine. p-Toluenesulphonyl chloride (620 mg., 2 equivalents) was added to the pyridine solution, which was then kept at 0° for 4 days.

The reaction solution, which was a bright red colour at this point was poured onto ice, and chloroform extracted. The chloroform extract was washed with ice-cold water, dried over anhydrous sodium sulphate and then evaporated to dryness at room temperature, in vacuo to yield a very viscous, light brown tar (935 mg.) I.R. ($CHCl_3$) 3430 $cm.^{-1}$ (OH), 1600 $cm.^{-1}$ (aromatic C-H) 1490 $cm.^{-1}$ (tosylate) 1360 $cm.^{-1}$ (tosylate) and 1170 $cm.^{-1}$ (tosylate). The peaks at 1490, 1360 and 1170 $cm.^{-1}$ were not present at the same frequencies in an infrared spectrum ($CHCl_3$) of p-toluenesulphonyl chloride.

The light brown tar resisted all attempts to purify it further.

ATTEMPTED ELLIMINATION OF p - TOLUENE-SULPHONIC ACID FROM DESOXIDOANNOTININEDIOL TOSYLATE.

A. With potassium hydroxide and methanol.

The oily tosylate (90 mg.) was dissolved in a minimum of a solution of methanolic potassium hydroxide. The red reaction solution was refluxed overnight and then evaporated to dryness at 60° under reduced pressure to yield a yellow solid residue. The residue was chloroform extracted and the extract washed with concentrated aqueous ammonia and then with water. After the extract had been dried over anhydrous sodium sulphate, evaporation of the chloroform at 60° under reduced pressure gave a light brown oil (85 mg.) I.R. ($CHCl_3$) 3430 cm.$^{-1}$ (OH), 1600 cm.$^{-1}$ (aromatic C-H), 1490 cm.$^{-1}$ (tosylate) and 1360 cm.$^{-1}$ (tosylate).

B. With pyridine.

A solution of the oily tosylate (97 mg.) in a minimum of pyridine was refluxed overnight. The light yellow reaction solution was evaporated to dryness at 60° under reduced pressure to yield a brown oil. A chloroform solution of the brown oil was washed with concentrated ammonium hydroxide and then water. The chloroform solution was dried over anhydrous sodium sulphate and evaporated to dryness to yield a pale yellow oil, whose infrared spectrum ($CHCl_3$) still exhibited peaks at 1600, 1490 and 1360 cm.$^{-1}$, that were characteristic of a tosylate.

C. With dimethyl sulphoxide.

Nace's method (36) was tried.

A solution of the red, oily tosylate (230 mg.) in dimethyl sulphoxide was heated on the steam bath, using a condenser that was fitted with a calcium chloride guard tube for 5 hours.

The red reaction solution was extracted with light petroleum (b.p. 60°-80°). Evaporation of the petroleum extract did not yield any material. Hence, the reaction solution was added to ice and the resulting solution was chloroform extracted. The chloroform extract was washed with dilute, aqueous sodium bicarbonate and then water. Anhydrous sodium sulphate was used to dry the extract which was then evaporated to dryness at 60° under reduced pressure to yield a light brown oil. The infrared spectrum ($CHCl_3$) of the oil still exhibited peaks at 1600, 1470, and 1360 $cm.^{-1}$, that were characteristeric of a tosylate.

D. With potassium hydroxide and ethanol.

Tosylate (170 mg.) from the previously attempted ellimination reactions (A, B and C) was dissolved in ethanolic potassium hydroxide and the solution was then refluxed for 36 hours. The reaction solution was evaporated to dryness at 60° under reduced pressure to yield a brown oil, that was then dissolved in water. The resulting aqueous solution was chloroform extracted and the extract washed with water and then dried over anhydrous sodium sulphate. Evaporation of the extract to dryness at 60° under reduced pressure yielded a brown oil, (63 mg.) I.R. ($CHCl_3$) 3480 $cm.^{-1}$ (OH), 3340 $cm.^{-1}$ (OH), 1665, 1640, 1595, 1570, 902 and 848 $cm.^{-1}$.

TREATMENT OF ANNOTININE, I, WITH TRIPHENYLPHOSPHINE. The method used was that of Wittig and Haag (19).

A. In an inert solvent.

A mixture of annotinine (620 mg., 1 equivalent) and triphenylphosphine (1020 mg., 2 equivalents) was dissolved in a high boiling inert solvent. p-Chlorotoluene (60 ml.) was used. The colourless reaction solution was refluxed at 175° for one hour under an atmosphere of nitrogen.

A preliminary investigation of the basicity of triphenylphosphine showed that dilute hydrochloric acid (0.1N) would extract 25% of the triphenylphosphine from a chloroform solution, but that if the acid were 0.05 N in strength, practically no triphenylphosphine was extracted from a chloroform solution. Therefore the reaction solution was extracted with dilute hydrochloric acid (0.05N). The aqueous acidic solution was then basified with dilute ammonium hydroxide and then chloroform (2 l.) extracted. The extract was dried over anhydrous sodium sulphate and evaporated to dryness at 60° under reduced pressure to yield unchanged annotinine (611 mg., 98.5% recovery) m.p. and I.R. ($CHCl_3$).

B. Fusion.

A mixture of annotinine (520 mg., 1 equivalent, m.p. 232°) and triphenylphosphine (427 mg., 1 equivalent m.p. 73°) under an atmosphere of nitrogen was melted (approximate m.p. 120°) in a paraffin wax bath. The reaction solution was then heated to 200° and kept at that temperature for 2 hours. However, most of the triphenylphosphine in the reaction solution sublimed from the reaction vessel to the condenser. The sublimate was identified by its m.p. (73°, lit. 73°) and its infrared spectrum in chloroform, which was identical with that

of an authentic sample. The sublimate was therefore returned to the reaction solution which was then kept at 135° for 27 hours.

The reaction mixture was cooled and dissolved in chloroform. Dilute hydrochloric acid (0.05 N) extraction of the chloroform solution yielded a colourless aqueous extract. The acidic aqueous extract was basified and then chloroform (2 l.) extracted. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness at 60° under reduced pressure to yield unchanged annotinine (463 mg., 89% recovery).

C. Sealed tube.

A mixture of annotinine (30 mg., 1 equivalent) and triphenylphosphine (25 mg., 1 equivalent) was heated in a sealed tube for 90 minutes at 225° . The light brown solid that was obtained was worked up in exactly the same way as the reaction mixture of method 'B'. Unchanged annotinine (25 mg., 83% recovery) was obtained.

Higher temperatures were tried, but with the same result.

HYDROGENOLYSIS OF ANNOTININE, I. The method used was that of MacLean(20).

Raney nickel (50 mg.) that had been freshly prepared by a method given in "Organic Syntheses" (14) was added to a solution of annotinine (160 mg.) in methanol (10 ml.). The mixture was heated for 40 hours, at a temperature of 170° , in an atmosphere of hydrogen, at a pressure of 2100 p. s.i.g.

The Raney nickel was removed by filtration and the filtrate

was then evaporated to dryness at 60° under reduced pressure to yield a brown oil. The infrared spectrum (chloroform) of the oil showed that it did not consist of annotinine, since there were peaks at 3450, 1774, and 1600 cm.$^{-1}$.

A solution of the oil in an ether-methylene chloride (5:1) mixture was chromatographed on basic alumina (3.5 g.). Two main fractions were eluted. With ether-chloroform (5:1) a light brown oil (80 mg.) was obtained, I.R. ($CHCl_3$) 3420, 1780, 1668 and 1644 cm.$^{-1}$, while elution with methylene chloride yielded a dark brown oil (44 mg.), I.R. ($CHCl_3$) 3410, 1782, 1668 and 1638 cm.$^{-1}$. Neither oil could be crystallised. Therefore, the oils were individually dissolved in acetone and a drop of dilute hydrochloric acid was then added. Evaporation of the solvent at 60° under reduced pressure, in each case, yielded a very dark brown oil. These oils could not be crystallised.

The experiment was repeated at 150°, for 35 hours, using hydrogen at 1300 p. s.i.g. A purer product was not obtained.

EPIDESOXIDOANNOTININEDIOL EPOXIDE HYDROBROMIDE. Crude *epi* desoxidoannotininediol epoxide (560 mg., 1.49 equivalents), obtained by hydride reduction of methyl *epi*annotinate, was added to a solution of hydrogen bromide (190 mg., 1.79 equivalents) in ether. Evaporation of the ether at 30° under reduced pressure yielded a black oil. The oil was well washed with acetone and a pale yellow, oily residue, which solidified on scratching, was obtained.

Crystallisation of the solid from a mixture of methanol and acetone yielded white prisms (529 mg., 73% yield) of <u>epi</u>desoxidoannotininediol epoxide hydrobromide. Twelve recrystallisations yielded an analytically pure sample m.p. 288° (dec.) I.R. (nujol), 3380 cm.$^{-1}$ (OH), 3300 cm.$^{-1}$ (OH), 2860 cm.$^{-1}$ ($-\overset{+}{N}-H$) and 2640 cm.$^{-1}$ ($-\overset{+}{N}-H$). (See spectrum, I.R. -4) Analysed as $C_{16}H_{26}O_3NBr$. Calculated:- C, 53.33% and H, 7.28%. Found: C, 53.38% and H, 7.29%. An analysis of the -O -Me content of the salt showed that there were none present.

RECONVERSION OF <u>EPI</u>DESOXIDOANNOTININEDIOL EPOXIDE HYDROBROMIDE TO THE FREE BASE. An aqueous solution of crystalline <u>epi</u>desoxidoannotininediol epoxide hydrobromide (73 mg.) was basified with concentrated ammonium hydroxide and was then chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a cream coloured solid (54 mg., 98% yield). The infrared spectrum (chloroform) of the solid was identical with the infrared spectrum of an authentic sample of <u>epi</u>desoxidoannotininediol epoxide.

<u>EPI</u>DESOXIDOANNOTININEDIOL EPOXIDE HYDROCHLORIDE. Concentrated hydrochloric acid (0.12 ml., 1.2 equivalents of hydrogen chloride) was added to a solution of white, greasy <u>epi</u>desoxidoannotininediol epoxide (330 mg., 1 equivalent) in acetone. The solution was shaken and then evaporated to dryness at 60° under reduced pressure to yield a green oil (350 mg.). Benzene

and 98% ethanol were added to the oil and the resulting solution was evaporated to dryness. The green oil was again obtained. The oil was dissolved in methanol, filtered and the filtrate was evaporated to dryness to yield a green oil (300 mg.). The green oil was crystallised from methanol - acetone as white rhomboids (115 mg., 31% yield). Six re-crystallisations yielded analytically pure colourless rhomboids of epidesoxidoannotininediol epoxide hydrochloride, m.p. 284° (dec.), I.R. (nujol) 3380 cm.$^{-1}$ (OH), 3270 cm.$^{-1}$ (OH) and 2670 cm.$^{-1}$ ($-N^{+}-H$), (spectrum I.R. -5). The rhomboids analysed as $C_{16}H_{26}O_{3}NCl$. Calculated:- C, 60.85%; H, 8.11%; O, 15.20%; N, 4.43% and Cl, 11.23%. Found:- C, 60.24%; H, 8.16%; O, 15.38%; N, 4.33% and Cl, 11.72%.

ATTEMPTED ACETYLATION OF EPIDESOXIDOANNOTININEDIOL EPOXIDE.

A solution of epides-oxidoannotininediol epoxide (90 mg.) in pyridine and acetic anhydride was kept overnight at room temperature and then was evaporated to dryness at room temperature, *in vacuo*, to yield a brown oil. An aqueous solution of the oil was basified with concentrated ammonium hydroxide and then was chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and was then evaporated to dryness at 30° under reduced pressure to yield a brown oil (115 mg.). The infrared spectrum (chloroform) did not exhibit any peaks that are characteristic of a hydroxyl group, but did exhibit peaks at 1732 cm.$^{-1}$ and 1260 cm.$^{-1}$, that are characteristic of an acetate group.

The acetate was not obtained in a crystalline form.

ATTEMPTED ACETYLATION OF ANNOTININETRIOL, XXXVI. A solution of annotininetriol (120 mg.) in pyridine and acetic anhydride was kept overnight at room temperature and was then evaporated to dryness at room temperature, in vacuo, to yield a brown oil. An aqueous solution of the oil was basified with concentrated ammonium hydroxide and then was chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30^{0} under reduced pressure, to yield a brown oil, (135 mg.). The infrared spectrum (chloroform) exhibited peaks at 1732 and 1255 $cm.^{-1}$, that are characteristic of an acetate group.

The acetate was not obtained in a crystalline form.

ANNOTININETETROL. Tetrahydrofuran (125 ml.) was distilled from a mixture of potassium metal and sodium hydroxide pellets into a dry flask that contained lithium aluminium hydride. (440 mg., 17 equivalents). A solution of pure annotinine hydrate (200 mg., 1 equivalent) in absolute benzene (25 ml.) was added to the flask. The reaction mixture was well stirred and refluxed for 3 days using a condenser that was fitted with a calcium chloride guard tube.

Water (0.44 ml.), 15% aqueous sodium hydroxide (0.44 ml.) and then water (0.44 ml.) were cautiously added to the reaction mixture. The resulting aluminium hydroxide was filtered and washed with methylene chloride (1 l.). The benzene filtrate and the methylene chloride washings were combined, dried over anhydrous sodium sulphate and then evaporated to dryness at

30° under reduced pressure, to yield a white solid (210 mg.). An ethereal solution of the white solid was reduced to a quarter of its original volume, scratched and then cooled, to yield a cream coloured amorphous solid (6mg.). The solid did not melt below 300° and did not exhibit any peaks in the carbonyl region of the infrared spectrum ($CHCl_3$) but showed two peaks at 3670 and 3450 $cm.^{-1}$ that were characteristic of a hydroxyl group.

Further crystals were not obtained, either from ether or any other possible crystallising medium.

Evaporation of the mother liquors to dryness at 30° under reduced pressure yielded a pale yellow oil. A small volume of benzene and ether (1:1) was added to the oil, which was then vigorously scratched. The oil solidified as a cream coloured solid (120 mg.) m.p. 188° -189° , which was washed with ice-cold acetone to yield a white solid (107 mg., 53% yield). This solid still could not be crystallised and oiled out even when scratched. The oil solidified in contact with benzene and the ensuing solid was sublimed at 205° , at a pressure of 0.02 m m. The white sublimate (m.p. 190° -191° , I.R. ($CHCl_3$) 3670 and 3420 $cm.^{-1}$) that was obtained sublimed unchanged. (For spectrum see I.R. -6) It analysed as $C_{16}H_{27}O_4N$. Calculated:- C, 64.59%; H, 9.16%; O, 21.52% and N, 4.71%. Found:- C, 65.73%; H, 9.19%; O, 20.86% and N, 4.62%.

ATTEMPTED ACETYLATION OF ANNOTININETETROL. The residues (70mg.) from the preparation of annotininetetrol were dissolved in

acetic anhydride (5 ml.). Pyridine (3 ml.) was added to the solution, which was then kept overnight at room temperature.

The solvent was evaporated from the red reaction solution at 40°, _in vacuo_, to yield a brown, viscous oil (170 mg.), I.R. ($CHCl_3$) 1730, 1670, 1650 and 1250 cm.$^{-1}$. A solution of the brown oil in methylene chloride was chromatographed on neutral "Woelm" alumina (4 g., grade III). A pale yellow oil (37 mg.) whose infrared spectrum ($CHCl_3$) exhibited peaks at 1730 and 1247 cm.$^{-1}$, but which did not show any other carbonyl peaks, or any hydroxyl peaks, was eluted with chloroform-methanol (50:1). The oil could not be crystallised.

THE ACTION OF SODIUM METHOXIDE ON ANNOTININE.

A. A methanolic solution (500 ml.) of annotinine (5.280 g.) and sodium methoxide (5.250 g.) was kept at room temperature in a flask fitted with a calcium chloride guard tube for 13 days. The reaction solution was evaporated to dryness at 30° under reduced pressure to yield a white grease. Water (1 l.) was added to the grease, which partially dissolved. The insoluble white solid that remained was filtered off, and the filtrate continuously extracted with ether. Removal of the ether, from the extract that was obtained after one day, yielded a colourless solid (2.2 g.). Ether was added to the material and the resulting mixture was refluxed for 60 minutes and then filtered. The filtrate was evaporated to dryness at 30° under reduced pressure to yield a mixture of methyl _epi_annotinate and annotinine hydrate (601 mg.), I.R. ($CHCl_3$) 3650 cm.$^{-1}$ (OH, non-bonded),

3500 $cm.^{-1}$ (OH, bonded), 1780 $cm.^{-1}$ (γ lactone) and 1730 $cm.^{-1}$ (-COO Me).

Two further days of continuous extraction with ether yielded a pale yellow gum, that consisted of an ether soluble solid (3.003 g.) and a chloroform soluble yellow grease (184 mg.). The ether soluble solid was annotinine hydrate, m.p. 212°-218° (lit. 220°-224°), whose infrared spectrum ($CHCl_3$) was identical to that of an authentic sample. The yellow grease was a mixture of methyl <u>epi</u>annotinate and annotinine hydrate, m.p. 149°, I.R. ($CHCl_3$) 3510 $cm.^{-1}$ (OH), 1775 $cm.^{-1}$ (γ lactone) and 1730 $cm.^{-1}$ (-COO Me).

The mixture of annotinine hydrate and methyl epiannotinate was dissolved in methylene chloride. The methylene chloride solution was chromatographed on 'Woelm', grade III, neutral alumina (5 g.). Annotinine hydrate (123 mg.) was eluted with methylene chloride and methyl <u>epi</u>annotinate (52 mg., 7% yield) was eluted with chloroform.

The total yield of annotinine hydrate was 3.126 mg. (56% yield).

The water insoluble, white solid that was obtained at the start of the work up, showed absorption in the infrared spectrum (nujol) at 3660, 3450 and 1620 $cm.^{-1}$ and did not melt below 300°. The infrared absorption at 1620 $cm.^{-1}$ indicated the presence of a sodium salt of a hydroxycarboxylic acid.

Hydrogen bromide was passed into a methanolic solution of methyl epiannotinate until the solution was acidic (pH 4).

Colourless microcrystals (168 mg.) precipitated out of the solution. After 5 recrystallisations from methanol-acetone (2:1), analytically pure white prisms of annotinine hydrate hydrobromide were obtained. They did not melt below 300° and showed peaks in the infrared (KBr) at 3340 cm.$^{-1}$ (OH), 3200 cm.$^{-1}$ (OH) and 1775 cm.$^{-1}$ (γ lactone). The crystals analysed as $C_{16}H_{24}O_4NBr$, Calculated:- C, 51.41%; H, 6.46%; O, 17.10%; N, 3.75% and Br, 21.35%. Found:- C, 51.41%; H, 6.52%; O, 17.23%; N, 3.74% and Br, 21.81%.

B. A mixture of annotinine (620 mg., 1 equivalent) and sodium methoxide (1.230 g., 10 equivalents) was dissolved in absolute methanol (156 ml.) and the ensuing solution was refluxed for 15 hours using a condenser that was fitted with a calcium chloride guard tube. The reaction solution was evaporated to dryness at 60° under reduced pressure to yield a white semi-solid. An aqueous solution of the white semi-solid was continuously extracted with benzene for one day. The extract was dried over anhydrous magnesium sulphate and was then evaporated to dryness at 30° under reduced pressure to yield white solid methyl epiannotinate (110 mg., 16% yield).

After 2 days of continuous extraction with benzene, annotinine (250 mg., 40% recovery) was obtained and after 7 days annotinine hydrate (150 mg., 23% yield) was obtained.

The poor yields in both experiments are probably due to the formation of amino acids.

REACTION OF ANNOTININE WITH POTASSIUM METHOXIDE.

A. Annotinine (3.330 g.) was dissolved in a solution of potassium

(3.6 g., 1.194 g. per g. of annotinine, 1.8 g. per 100 ml. of methanol) in absolute methanol (200 ml.). The reaction solution was kept at room temperature, in a flask that was fitted with a calcium chloride guard tube, for 3 days.

Most of the methanol was evaporated from the reaction solution at room temperature under reduced pressure, to yield an orange oil. The last few drops of methanol were removed *in vacuo* to yield a cream coloured solid. Ice cold water (20ml.) was added to the solid which partially dissolved. Filtration of the mixture yielded ester M as a white solid. (2.4 g., 71% yield).

The aqueous, basic filtrate was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield white solid methyl annotinate (1.087 g., 29% yield). Recrystallisation from anhydrous ether yielded cream coloured microcrystals, m.p. 129°-134° (lit. 132°-134°). The solid exhibited an infrared spectrum that was almost identical to that of a sample of methyl *epi*-annotinate.

The ester M was completely insoluble in all hot and cold solvents except glacial acetic acid, and refluxing methanol. A mixture of the ester and methanol was refluxed for 30 minutes and then filtered. The volume of the filtrate was reduced to 25 ml. and then acetone (20 ml.) was added. The solution was cooled and white crystals (1.790 mg., 53% yield) were obtained. Seven recrystallisations from methanol -acetone

yielded an analytically pure sample, m.p. 285°-292° (dec.) I.R. (nujol) 3440 cm.$^{-1}$ (OH), broad band at 2600 - 2800 cm.$^{-1}$ (anomalous) and 1724 cm.$^{-1}$ (COO Me). (See I.R. -7). The fingerprint region of the spectrum was not similar to that of methyl epiannotinate. The ester was analysed as $C_{17}H_{25}O_4N$.
Calculated:- C, 66.42%; H, 8.20%; O, 20.82% and N, 4.55%
Found:- C, 66.28%; H, 8.16%; O, 21.79% and N, 3.12%.

B. Annotinine (2.461 g.) was dissolved in a solution of potassium metal (2.333 g.; 0.948 g. per g. of annotinine; 1.8 g. per ml. of methanol) in absolute methanol (120 ml.). The reaction solution was kept at room temperature, in a flask that was fitted with a calcium chloride guard tube, for 3 days.

Most of the methanol was evaporated off at 60° under reduced pressure and the remainder was removed at room temperature *in vacuo*, to yield a cream coloured powder. Water (50 ml.) at 10° was added to the powder, which partially dissolved. Filtration of the mixture yielded ester M, as a white solid (1.513 g., 56% yield m.p. 291°).

Continuous extraction with ether of the aqueous, basic filtrate yielded further ester M (30 mg.) m.p. 285° I.R. (nujol) 3450 and 1724 cm.$^{-1}$.

C. Annotinine (3.330 g.) was dissolved in a solution of potassium (3.140 g., 0.941 g. per g. of annotinine, 1.9 g. per 100 ml. of methanol) in absolute methanol (160 ml.). The reaction solution was kept at room temperature in a flask

that was fitted with a calcium chloride guard tube, for 3 days. The reaction was split into 3 portions:- (a) 400 ml., (b) 500 ml. and (c) 700 ml.

(a) The majority of the methanol was removed at 60° under reduced pressure and the remainder was evaporated at room temperature, in vacuo, to yield a pale yellow solid. The infrared spectrum (nujol) did not show any absorption between 1750 and 1800 $cm.^{-1}$. However, there were peaks at 1724 $cm.^{-1}$ and 1638 $cm.^{-1}$ Water (100 ml.) at 10° was added to the solid which partially dissolved. Filtration of the mixture yielded ester M, as a white solid (680 mg., 73% yield) m.p. 289°.

(b) The methanolic solution was evaporated to dryness, in a stream of purified nitrogen which was dried by passage over phosphorus pentoxide. A pale yellow solid was obtained. The infrared spectrum (nujol) of the solid was identical to that obtained in (a). Water(100 ml.) was added to the solid, which partially dissolved. Filtration of the mixture yielded ester M as a white solid (1.030 g., 89% yield) m.p. 290°.

(c) The methanolic solution was evaporated to dryness in a stream of nitrogen under reduced pressure to yield a pale yellow solid. Water (100 ml.) was added to the solid, which partially dissolved. Filtration of the mixture yielded ester M as a white solid (1.400 g., 86% yield) m.p. 290°.

D. The method used was that of MacLean(20).

Annotinine was dissolved in a freshly prepared solution of potassium methoxide and kept at room temperature for 3

days in a container that was fitted with a calcium chloride guard tube. The weights of annotinine and potassium metal per 100 ml. of absolute methanol, that gave methyl epiannotinate were respectively:- (a) 0.318 g. and 0.305 g. (1.4% yield); (b) 1.86 g. and 1.80 g. (31% yield) and (c) 0.80 g. and 1.90 g.(38% yield); (d) 1.83 and 1.83 g. (44% yield) and finally (e) 1.77 g. and 1.80 g. (61% yield).

The majority of the methanol was removed from the reaction solution at 30° under reduced pressure to yield a white semi-solid. Water (60 ml. per 100 ml. of methanol) was added to the semi-solid. In cases (a) and (e), methyl epiannotinate precipitated out of solution as a white solid, while in the other cases an aqueous solution was obtained that was ether extracted. The extract was washed with water, dried over anhydrous magnesium sulphate and then reduced in volume at 45° until white needles of methyl epiannotinate began to spontaineously come out of solution.

In all cases continuous extraction with ether of the basic aqueous solution did not yield any further methyl epiannotinate. However in all cases some annotinine hydrate was obtained, although a 100% recovery of material was not achieved in any of the experiments.

EPIMERISATION OF METHYL ANNOTINATE, XXXIV. Methyl annotinate (360 mg.) was dissolved in a solution of potassium methoxide (903 mg.) in the minimum of absolute methanol. The solution was kept for one day at room temperature in a flask that was

fitted with a calcium chloride guard tube and was then refluxed for 4 hours using a condenser that was fitted with a calcium chloride guard tube. The reaction solution was evaporated to dryness at 30° under reduced pressure to yield a white semi-solid. The white semi-solid was washed with methylene chloride (1 l.). Evaporation of the methylene chloride at 30° under reduced pressure yielded a yellow solid (440 mg.). Ether was added to the solid and the mixture was refluxed for 30 minutes and was then filtered. The volume of the filtrate was reduced until crystals came out of solution. The mixture was cooled and filtered. Crystalline annotinine hydrate (220 mg.) was obtained. The residue of the semi-solid that had been washed with methylene chloride was soxhlet extracted with methylene chloride. Evaporation of the methylene chloride gave a further yield of annotinine hydrate (230 mg.) (identified by its infrared spectrum).

TRIOL M, L. Ester M (360 mg.) was placed in a filter paper thimble and the thimble was placed in a soxhlet extractor, in which anhydrous benzene (200 ml.) was circulating. After 4 days, a solution of the ester M was obtained which was added dropwise to lithium aluminium hydride (1.020 g., 22 molar excess). The mixture was refluxed and stirred for 3 days, using a condenser that was fitted with a calcium chloride guard tube.

Water (1.02 ml.), 15% aqueous sodium hydroxide (1.02 ml.) and then water (1.02 ml.) again were cautiously added to the cold reaction mixture. The mixture was filtered and the

aluminium hydroxide was washed with methylene chloride (750 ml.). The benzene filtrate and the methylene chloride washings were combined, dried over anhydrous magnesium sulphate and then evaporated to dryness at 30° under reduced pressure to yield a white powder (140 mg.). Recrystallisation of the solid from acetone yielded a cream coloured solid, triol M, m.p. 130°-131°, I.R. (nujol) 3730 and 3425 cm.$^{-1}$, no carbonyl absorption. Further recrystallisations gave increasingly darker microcrystals. A small sample of triol M sublimed at 121° under 0.02 mm pressure, to yield a sublimate that melted at 112° and 130°. The remainder of the triol was dissolved in acetone and concentrated hydrochloric acid was added until the solution was just acidic. Poorly formed, colourless crystals immediately precipitated out of solution. Five recrystallisations from glacial acetic acid-acetone yielded analytically pure, colourless prisms of triol M hydrochloride, m.p. 292°-294°. I.R. (nujol) 3430 cm.$^{-1}$ (OH), 3330 cm.$^{-1}$ (OH)3230 cm.$^{-1}$, 2650 cm.$^{-1}$ ($-N^{+}-H$) and 2600 cm.$^{-1}$ ($-N^{+}-H$) (See I.R. -8). Analysed as $C_{16}H_{26}O_3NCl\,\frac{1}{3}H_2O$. Calculated C, 59.73%; H, 8.33%; O, 16.58%; N, 4.35%; and Cl, 11.03%. Found:- C, 59.78%; H, 8.25%; O, 16.78%; N, 4.24% and Cl, 10.64%.

The aluminium hydride that had been washed with methylene chloride was placed in the thimble of a soxhlet extractor. Extraction with acetone for 2 days yielded a colourless solution, to which one drop of concentrated hydrochloric acid was

added. The solution immediately turned a bright red colour. Concentration of the red solution yielded yellow prisms which were washed with ice-cold methanol, to yield colourless prisms (120 mg.) of the triol M hydrochloride, m.p. 289°.

The overall yield of crystalline triol M hydrochloride was 190 mg. (50%).

ATTEMPTED ACID ISOMERATION OF ESTER M, XXXVII.

A solution of ester M (20 mg.) in glacial acetic acid (20 ml.) was kept at room temperature for 26 hours. The solution was evaporated to dryness at 60° in vacuo, to yield a white semi-solid. The semi-solid was dissolved in acetone. Upon cooling the acetone solution, white prisms of ester M (15 mg.) crystallised out of solution. The infrared spectrum (nujol) of the prisms was identical to that given by an authentic sample of ester M.

ACTION OF MINERAL ACIDS ON ESTER M, XXXVII.

A. Dilute Hydrochloric Acid.

A solution of ester M (40 mg.) in hydrochloric acid (6N, 10 ml.) was refluxed for 10 hours.

Benzene and 98% ethanol were added to the colourless reaction solution. The solution was then evaporated to dryness at 60° under reduced pressure to yield an opaque oil (43 mg.). The oil was washed with ether. A solid white residue (40 mg., 90% yield) of acid M hydrochloride remained. The identity of the salt was found by comparing its infrared spectrum (nujol) with that of an authentic sample of acid M hydrochloride.

Recrystallisation of the salt 7 times from methanol-acetone yielded a pure sample of the compound, which analysed as $C_{16}H_{24}O_4NCl$. Calculated:- C, 58.26%; H, 7.33%; O, 19.40%; N, 4.25% and Cl, 10.75%. Found:- C, 57.98%; H, 7.37%; O, 19.50%; N, 4.45% and Cl, 10.62%.

B. Hydrogen chloride.

A methanolic solution of the ester M (325 mg.) was saturated with dry hydrogen chloride and was then refluxed for 14 hours using a condenser that was fitted with a calcium chloride guard tube.

The colourless reaction solution was evaporated to dryness at 60° under reduced pressure to yield a pale blue solid (380 mg.). Crystallisation of the blue solid from methanol-acetone yielded white prisms of ester M hydrochloride (303 mg., 83% yield). The infrared spectrum of the prisms was identical to that of an authentic sample of ester M hydrochloride.

ACIDS M and N, XLI. A mixture of ester M (300 mg.) and 98% ethanol (150 ml.) was refluxed until solution had occurred. Then a solution of barium hydroxide (2 g.) in water (75 ml.) was added. The reaction solution was refluxed for 24 hours, using a condenser that was fitted with an ascarite guard tube.

The solution was saturated with carbon dioxide and the barium carbonate that was precipitated was filtered off. Further carbon dioxide was passed into the filtrate, which

remained clear. Absolute benzene (200 ml.) was added to the filtrate and the resulting azeotropic mixture was evaporated in a stream of nitrogen at 80° under reduced pressure. White prisms (151 mg., 53% yield) of acid M crystallised out of the solution before all the solvent had been removed. Complete removal of the solvent yielded acid N (75 mg., 26% yield) as a cream coloured solid.

Recrystallisation of acid M, 6 times, from water - benzene - 98% ethanol (1:1:1) yielded white, analytically pure prisms m.p. 329° (uncorr.), I.R. (nujol) 3570 cm.$^{-1}$ (OH), 3510 cm.$^{-1}$ (OH), a broad band between 2500 and 3000 cm.$^{-1}$ ($-\overset{+}{N}$-H) 1658 cm.$^{-1}$ (anomalous) and 1565 cm.$^{-1}$ ($-COO^{-}$). (See I.R.-9). Analysis: calculated for $C_{16}H_{23}O_{4}N$, - C, 65.52%; H, 7.90%; O, 20.82%; and N, 4.78 %. Found:- C, 65.65%; H, 7.96%; O, 22.04% and N, 4.83%.

Recrystallisation of acid N from methanol-acetone yielded white prisms, m.p. 272°-302° (dec.) I.R. (nujol) 3480 cm.$^{-1}$ (OH) 3240 cm.$^{-1}$ (OH or $-\overset{+}{N}$-H) 2400 - 2500 cm.$^{-1}$ ($-\overset{+}{N}$-H) and 1585 cm.$^{-1}$ ($-COO^{-}$).

A solution spectrum of either of the acids could not be obtained.

ACID M HYDROCHLORIDE, XLII.

A. Acid M (25 mg., 1 equivalent) was dissolved in a minimum of boiling water. Concentrated hydrochloric acid (0.02 ml., 2.4 equivalents) was added to the aqueous solution and then absolute benzene and 98% ethanol were added. The azeotropic mixture was evaporated at 60° under reduced pressure to yield a

pale yellow solid.(27 mg.). A recrystallisation of the yellow solid from methanol-acetone yielded white stars of acid M hydrochloride (17 mg.), m.p. 308° (dec.), I.R. (nujol) 3480 $cm.^{-1}$ (OH), 3400 $cm.^{-1}$, 3300 $cm.^{-1}$, 2700 $cm.^{-1}$ ($-\overset{+}{N}-H$), 2620 $cm.^{-1}$ ($-\overset{+}{N}-H$), 1719 $cm.^{-1}$ (-COOH) and 1632 $cm.^{-1}$ (anomalous).

B. Acid N (11 mg.) was dissolved in aqueous methanol and concentrated hydrochloric acid (0.01 ml., 2 equivalents) was then added to the aqueous methanolic solution. Benzene and 98% ethanol were added to the reaction solution which was then evaporated to dryness at 60° under reduced pressure to yield a white solid. Crystallisation of the white solid from methanol-acetone yielded white stars of acid M hydrochloride (8 mg.), m.p. 308° (dec.). The infrared spectrum (nujol) was identical with that exhibited by a genuine sample of acid M hydrochloride.

Another sample of acid M hydrochloride was prepared. This sample was recrystallised 6 times from methanol-acetone to yield analytically pure white prisms. Analysed as $C_{16}H_{24}O_4NCl$. Calculated:- C, 58.26%; H, 7.33%; O, 19.40%; N, 4.25% and Cl, 10.75%. Found:- C, 57.98%; H, 7.37%; O, 19.50%; N, 4.45% and Cl, 10.62%. (See I.R. -10).

METHYLATION OF ACID M. An ethereal solution of diazomethane was added to a solution of acid M (45 mg.) in aqueous methanol until a pale yellow colour persisted for 3 minutes. The solution was evaporated to dryness at 60° under reduced pressure to yield a white solid that crystallised from methanol-acetone

as white rhomboids (21 mg.). The rhomboids were ester M, (identified by m.p. and I.R. (nujol).

METHYLATION OF ACID N. An aqueous methanolic solution of acid N (7 mg.) was treated with an ethereal solution of diazomethane until the solution was a yellow colour for 3 minutes. The reaction solution was evaporated to dryness at 60° under reduced pressure, to yield a white solid that crystallised from methanol-acetone as white rhomboids of ester M (4 mg.), (identified by m.p. and I.R. (nujol)).

CONVERSION OF ESTER M TO ESTER M HYDROCHLORIDE, LI. Dilute hydrochloric acid (6N, 0.55 ml., 27 equivalents) was added to a suspension of ester M (30mg., 1 equivalent) in acetone. Benzene and 98% ethanol were added to the resulting solution. The azeotropic mixture was removed at 60° under reduced pressure to yield white cubes (33 mg., 99.5% yield) of ester M. hydrochloride, m.p. 295° (dec.) I.R. 3580 $cm.^{-1}$ (OH), 3300 $cm.^{-1}$, 2570 ($-N^{+}-H$) and 1703 $cm.^{-1}$ (-COO Me).

RECONVERSION OF ESTER M HYDROCHLORIDE TO ESTER M. Ester M hydrochloride was dissolved in a mixture of dilute ammonium hydroxide (1N, 5 ml.) and chloroform. The 2 solvents were shaken and separated. The chloroform solution was evaporated to dryness at 30° under reduced pressure to yield a white powder. The white powder was identified as ester M by its m.p. and I.R. (nujol).

ACETYLATION OF ESTER M.

A. Ester M (220 mg.) was mixed with acetic anhydride (30 ml.)

and pyridine (30 ml.). The ester did not dissolve and so the mixture was stirred and heated for 2 hours at 100°. A pale yellow solution was obtained.

The reaction solution was evaporated to dryness in a stream of nitrogen at 60° under reduced pressure to yield a brown oil. A chloroform solution of the oil was shaken with dilute ammonium hydroxide (0.5 N.), dried over anhydrous sodium sulphate and evaporated to dryness at 60° under reduced pressure to yield a brown oil (220 mg.). Continuous extraction with ether of the ammonium hydroxide solution yielded a brown oil (8 mg.).

A chloroform solution of the 2 brown oils was chromatographed on neutral "Woelm" alumina (grade I, 3 g.). Elution with ether yielded a pale yellow oil (102 mg.), I.R. ($CHCl_3$) 1736, 1730 and 1252 cm.$^{-1}$. Dilute hydrochloric acid (1 N, 0.3 ml.) was added to a solution of the pale yellow oil in acetone. Evaporation of the acetone at 60° under reduced pressure yielded a white grease that crystallised from methanol-acetone as cream coloured microcrystals m.p. 264° (dec.) I.R. (nujol) 3310 cm.$^{-1}$ (OH), 2580 cm.$^{-1}$ ($-\overset{+}{N}-H$), 1760 cm.$^{-1}$, 1736 and 1228 cm.$^{-1}$. An analytically pure sample could not be obtained of what is presumably the monoacetate.

B. A mixture of ester M (320 mg.) and acetic anhydride (50 ml.) was stirred and heated at 100°, until a solution was obtained. Pyridine (30 ml.) was added to the solution which was then stored at room temperature for 11 days.

The pale yellow reaction solution was evaporated to dryness at 60° under reduced pressure to yield a brown oil. A chloroform solution of the brown oil was shaken with dilute ammonium hydroxide, dried over anhydrous sodium sulphate and added to an equal volume of acetone. Concentrated hydrochloric acid (1 ml.) was added to the acetone and chloroform solution, which was then evaporated to dryness at 60° under reduced pressure to yield a yellow solid. A chloroform solution of the yellow solid was chromatographed on neutral "Woelm" alumina (grade I, 110 g.) Ethyl acetate elution yielded a pale yellow viscous oil (250 mg., 62% yield). Eight crystallisations of the viscous oil from ethyl acetate-ether yielded microcrystals which were tinted yellow. The last four recrystallisations did not produce any change in the melting point of the crystals, which was 226.5°. The infrared spectrum (nujol) exhibited peaks at 1746 cm.$^{-1}$ (-COO Me or acetate), 1738 cm.$^{-1}$ (-COO Me or acetate) and 1237 cm.$^{-1}$ (acetate). (See I.R. -11). The compound analysed as the diacetate, $C_{21}H_{29}O_{6}N$: Calculated C, 64.43%; H, 7.47%; O, 24.52%; and acetyl, 22.00%. Found:- C, 69.77%; H, 7.10%; O, 22.90% and acetyl, 19.92%.

ATTEMPTED LACTONISATION OF ACID M. A mixture of acid M (40 mg.), p-toluenesulphonic acid and absolute benzene was placed in a flask that was fitted with a water separator and a reflux condenser, which was fitted with a calcium chloride guard tube. The mixture was refluxed for 3 days. There

was no water in the water separator.

The reaction mixture was shaken with chloroform and then filtered. Evaporation of the filtrate at 30° under reduced pressure yielded an intractable yellow oil. I.R. ($CHCl_3$) 1729, 1641 and 1607 cm^{-1}.

The chloroform insoluble material was dissolved in boiling water and was chloroform extracted. The chloroform extract was washed with dilute aqueous ammonium hydroxide, dried over anhydrous sodium sulphate and then evaporated to dryness at 60° under reduced pressure, to yield a white powder. The infrared spectrum (nujol) of the white powder was identical to that of the starting material.

ANNOTININEDIOL, LX. The method of Marion (38) was used.

A solution of annotinine (905 mg.) in 25% sulphuric acid (5 ml.) was gently refluxed for 24 hours and was then stored at room temperature for 2 days.

The reaction solution was basified with concentrated ammonium hydroxide and then extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness to yield a brown oil.

A chloroform solution of most of the oil was chromatographed on basic alumina (18 g.). Elution with chloroform-benzene (9:1) yielded a pale yellow solid (68 mg.) whose infrared spectrum ($CHCl_3$) was identical to that of a sample of annotinine hydrate. Chloroform-methanol (9:1) elution

yielded a pale yellow solid (530 mg.). The solid did not melt below 300° and showed peaks in the infrared spectrum ($CHCl_3$) at 3450 and 1778 cm.$^{-1}$. Chloroform-methanol (1:1) elution yielded a dark yellow solid (63 mg.) that did not melt below 300° and showed peaks in the infrared spectrum at 3650, 3450 and 1781 cm.$^{-1}$.

A drop of dilute hydrochloric acid (6 N.) was added to an acetone solution of the pale yellow and the dark yellow solids. A brown solid instantly precipitated from solution. Crystallisation of the solid from methanol-acetone yielded yellow needles of annotininediol hydrochloride (649 mg., 60% yield), I.R. (nujol) 3520 cm.$^{-1}$ (OH), 3485 cm.$^{-1}$, 3320 cm.$^{-1}$, 2600 - 2750 cm.$^{-1}$ ($\overset{+}{-N-H}$) and 1781 cm.$^{-1}$ (δ lactone). The infrared spectra ($CHCl_3$) of annotinine hydrochloride and annotinine hydrate hydrochloride were not superimposable on the spectrum of annotininediol hydrochloride. The m.p. was initially 242° - 245° (lit. 292° -294°), but the needles melted at 290° after they had been dried for 16 hours at 130°.

The yellow solid material (256 mg.) that had been insoluble in chloroform and so had not been chromatographed was crystallised from methanol-acetone as white prisms (228 mg., 24% yield) of annotininediol, m.p. 232°, I.R. (nujol) 3470 cm.$^{-1}$ (OH), 3230 cm.$^{-1}$ and 1786 cm.$^{-1}$ (γ lactone).

ANNOTININE HYDROCHLORIDE LXII. Dilute hydrochloric acid (0.02 ml.) was added to a solution of annotinine (11 mg.) in acetone.

The solution was evaporated to dryness at 60° under reduced pressure to yield a pale yellow solid. The pale yellow solid was washed with ice-cold acetone to yield annotinine hydrochloride (9 mg.) as a white solid, m.p. 242° - 245°, I.R. (nujol) 3400 - 3450 cm.$^{-1}$ (OH), 2600 cm.$^{-1}$ (-$\overset{+}{N}$-H) and 1785 cm.$^{-1}$ (γ lactone).

ANNOTININE HYDRATE HYDROCHLORIDE, Dilute hydrochloric acid (0.05 ml.) was added to a methanolic solution of annotinine hydrate (18 mg).

The solution was evaporated to dryness at 60° under reduced pressure to yield a pale yellow solid. The pale yellow solid was washed with ice-cold acetone, to yield annotinine hydrate hydrochloride (15 mg.) as a white solid. I.R. (nujol) 3325 cm.$^{-1}$ (OH), 3150 cm.$^{-1}$ (-$\overset{+}{N}$ -H) and 1781 cm.$^{-1}$ (γ lactone). The salt did not melt below 300°.

ANNOTININEDIOL AND POTASSIUM METHOXIDE. Annotininediol (55 mg., 0.19 millimoles) was dissolved in a refluxing solution of potassium (49 mg., 0.70 millimoles) in absolute methanol (2.5 ml.). A condenser that was fitted with a calcium chloride guard tube was used. After one hour, the solution was cooled and kept at room temperature for 11 days.

The reaction solution was evaporated to dryness at 30° under reduced pressure to yield a yellow solid. Ice cold water (1 ml.) was added to the solid, which instantly dissolved to give a pale yellow solution that was chloroform

extracted. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a white semi-solid (6 mg.), I.R. (film) 3200 - 3500, 1674 and 1570 $cm.^{-1}$.

The basic aqueous solution was continuously extracted with ether for 4 days. The ether was removed from the extract at 30° under reduced pressure to yield an aqueous solution. Benzene and 98% ethanol were added to the aqueous solution and the resulting azeotropic mixture was removed at 60° under reduced pressure to yield a yellow oil. (34 mg.) I.R. ($CHCl_3$) 3450 and 1732 $cm.^{-1}$.

Neither of the spectra obtained bore any similarity to a spectrum ($CHCl_3$ or nujol) of ester M or acid M or N.

ACTION OF POTASSIUM METHOXIDE ON ANNOTININE HYDRATE. Annotinine hydrate (295 mg., 1 equivalent) was dissolved in a solution of potassium (270 mg., 7 equivalents) in absolute methanol (22 ml.). The reaction solution was kept at room temperature for 3 days.

The solution was evaporated to dryness in a stream of nitrogen under reduced pressure to yield a yellow solid, I.R. (nujol) 3250, 3450, 1675 and 1580 $cm.^{-1}$. An aqueous solution (50 ml.) of the yellow solid was chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness at 30° under reduced pressure to yield a pale yellow oil, I.R. (film) 1748 $cm.^{-1}$. The oil was not purified further.

Continuous extraction with ether of the aqueous, basic solution yielded a colourless extract. The extract was washed with water, dried over anhydrous magnesium sulphate and then evaporated at 30^{o} under reduced pressure to yield a white powder. The white powder was annotinine hydrate (180 mg., 62% recovery).

METHYL EPIANNOTINATE, XXX AND POTASSIUM METHOXIDE.

A. Methyl epiannotinate (90 mg., 0.29 millimoles) was dissolved in a solution of potassium (84 mg., 22 millimoles) in absolute methanol (4.3 ml.). The colourless solution was kept at room temperature for 83 hours in a flask that was fitted with a calcium chloride guard tube, and then refluxed for one hour using a condenser that was fitted with a calcium chloride guard tube.

The reaction solution was evaporated to dryness at 40^{o} under reduced pressure to yield a yellow semi-solid. The semi-solid was kept in vacuo, at room temperature until it had solidified to a yellow solid. Ice-cold water (5 ml.) was added to the yellow solid which readily dissolved. The aqueous solution was ether extracted and the extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness at 39^{o} under reduced pressure to yield a white semi-solid. (48 mg.) I.R. ($CHCl_3$) 3660, 3490, 1780, (weak band) and 1730 $cm.^{-1}$.

The basic aqueous solution was continuously extracted with ether for 3 weeks. The extract was evaporated to dryness at 60^{o} under reduced pressure to yield a white viscous oil. The infrared spectrum ($CHCl_3$) of the viscous oil was identical to that of an authentic sample of annotinine hydrate.

B. A solution of methyl epiannotinate (240 mg., 0.78 millimoles) and sodium methoxide (1.2 g., 22 millimoles) in absolute methanol

(50 ml.) was refluxed for 94 hours, using a condenser that was fitted with a calcium chloride guard tube. (The methyl epianhydrotinate had been recrystallised twice from ether to ensure that it did not contain any ester M.)

The colourless reaction solution was evaporated to dryness at 60° under reduced pressure to yield a pale yellow oil, which solidified *in vacuo* after one hour. Ice-cold water (5 ml.) was added to the solid, which readily dissolved. The aqueous solution was ether extracted and the extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness at 30° under reduced pressure to yield a colourless oil. The infrared spectrum ($CHCl_3$) of the oil did not exhibit any carbonyl absorption.

ESTER M AND POTASSIUM METHOXIDE. Ester M (150 mg., 0.49 millimoles) was added to a solution of potassium (148 mg., 3.6 millimoles) in absolute methanol (7.2 ml.). The ester did not dissolve at all at room temperature. The mixture was therefore refluxed using a condenser that was fitted with a calcium chloride guard tube. After 3 days, all of the ester had dissolved.

Most of the methanol was evaporated at 30° under reduced pressure and the remainder was evaporated at room temperature, *in vacuo*, to yield a yellow solid. An aqueous solution of the yellow solid was ether extracted and the extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness at 30° under reduced pressure to yield a yellow oil (30 mg.,). The oil was intractable. Its infrared spectrum ($CHCl_3$) was very poorly resolved but did

exhibit a weak band at 1742 $cm.^{-1}$, and hydroxyl bands were not present.

Continuous extraction with ether of the aqueous solution for 3 days yielded an intractable yellow, viscous oil (30 mg.), whose infrared spectrum ($CHCl_3$) was very poorly resolved. Weak bands were exhibited at 1774 and 1732 $cm.^{-1}$.

Continuous extraction with ether for 2 more days yielded a white powder (30 mg.) that decomposed in a wide range below 170°. The infrared spectrum (nujol) of the powder was very poorly resolved, but peaks were exhibited at 3300 - 3500 and 1736 $cm.^{-1}$.

QUALITATIVE REACTION OF PERIODIC ACID WITH ESTER M. Concentrated nitric acid (1 drop) and ester M (2 mg.) were added to a solution of periodic acid (3 mg.) in methanol (2 ml.). The resulting solution was kept at room temperature for 50 minutes. Silver nitrate solution (0.1 N, 1 drop) was then added to the first solution. The solution remained clear.

ACTION OF PERIODIC ACID ON ESTER M. Periodic acid (490 mg., 2.1 millimoles) and ester M (305 mg., 1 millimole) were dissolved in warm methanol (55 ml.). The resulting colourless solution was refluxed for 90 minutes, cooled and stored at room temperature overnight.

The pale yellow reaction solution was evaporated to dryness at 30° under reduced pressure to yield a pale yellow solid. An ethereal solution of the solid was treated with an

ethereal solution of diazomethane. Nitrogen was not evolved and the ethereal solution was reduced to a quarter of its original volume. A white crystalline solid that precipitated was filtered off. An aqueous solution of the crystalline solid was chloroform extracted. The extract was dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a pale yellow powder (160 mg.). The infrared spectrum (nujol) of the solid was identical to that of an authentic sample of ester M.

Crystals precipitated from the aqueous solution. These were filtered off and were crystals (70 mg.) of ester M. A further yield of ester M (45 mg.) was obtained by continuous extraction with ether of the aqueous filtrate for one day.

The overall recovery of ester M was 275 mg. (90%).

TIME CURVE OF THE REACTION OF PERIODIC ACID WITH ESTER M.

Periodic acid (25.4 mg.) and a solution of ester M (25.6 mg.) in concentrated nitric acid were added to a 100 ml. graduated flask. Water was then added until the volume of the solution was 100 ml. A blank was also prepared. Every 12 hours an aliquot (4 ml.) was taken from the solution and from the blank. Aqueous potassium iodide (20 mg., in 5 ml.) was added to each aliquot and the liberated iodine was titrated with standard sodium thiosulphate, using starch as the end point indicator.

After 4 days the blank had taken up as much periodic acid as had ester M.

THE TIME CURVE OF THE REACTION BETWEEN LEAD TETRAACETATE AND ESTER M. A solution(100 ml.) of ester M (24.6 mg., 0.080 millimoles) and freshly prepared lead tetraacetate (145 mg., 0.292 millimoles), in absolute glacial acetic acid was prepared. Similar solutions(100 ml.) were also prepared from (a) annotinine hydrate (26.3 mg., 0.090 millimoles) and (b) annotininediol (26.6 mg., 0.090 millimoles) and (c) a blank with lead tetraacetate (145 mg.) and absolute glacial acetic acid.

Every 12 hours an aliquot (4 ml.) was pipetted from each of the solutions and a mixture of potassium iodide (0.5 g.) and sodium acetate (5 g.) in water (25 ml.) was added to each aliquot. The amount of unreacted lead tetraacetate in each of the solutions was found by titrating the liberated iodine in each aliquot against a standard solution of sodium thiosulphate (0.0005 N) using starch as the end point indicator. After 84 hours, an equal, but small amount of lead tetraacetate had reacted in each of the solutions.

Water (5 ml.) was then added to each of the solutions. After 19 days no significant differences in the uptake of lead tetraacetate in any of the solutions were observed.

The experiment was repeated using ester M hydrochloride (17 mg.) annotininediol hydrochloride (17 mg), mannitol (17 mg.) and a blank that contained dilute hydrochloric acid (6 N,0.01ml.). The annotininediol hydrochloride solution required 8 days for complete consumption, the ester M solution 14 days, and the blank, 20 days.

ATTEMPTED CrO_3-PYRIDINE OXIDATION OF ESTER M. Boiling absolute pyridine (100 ml.) was added to ester M (290 mg.). The

resulting solution was cooled to 10° and then added dropwise, with stirring, to a solution of chromium trioxide (290 mg.) in absolute pyridine (100 ml.). The reaction solution was magnetically stirred for 4 days at room temperature.

A stream of nitrogen was passed through the dark brown reaction suspension under reduced pressure at room temperature to yield a dark brown solid. A mixture of ice and water was added to the solid and the resulting suspension was chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a dark brown solid (180 mg.). A solution of the brown solid in methanol-98% ethanol (1:1) was filtered through a small column of basic alumina (5 g.). The solution was not decolourised at all, so the solution was heated with a little animal charcoal, filtered and then evaporated to dryness at 30° under reduced pressure, to yield a pale green oil. Solidification of the oil occurred after a little acetone had been added, to yield a pale green solid (45 mg.) that did not melt below 300°. The infrared spectrum (nujol) of the solid was very poorly resolved. However the peaks that were exhibited were also given by the ester M. The solid did not sublime.

The aqueous suspension of chromium salts was basified with concentrated aqueous ammonium hydroxide and was then continuously extracted with ether for 3 days. The extract consisted of a yellow aqueous portion and a yellow ethereal portion. The ether was evaporated at 30° under reduced

pressure. Benzene and 98% ethanol were then added to the aqueous solution and the water was azeotropically removed at 30° under reduced pressure to yield a pale yellow powder. The powder was crystallised from methanol and acetone to yield pale yellow prisms of ester M (166 mg., 58% recovery).

CHROMIUM TRIOXIDE -ACETIC ACID OXIDATION OF ESTER M.

A. Room temperature.

An orange solution of chromium trioxide (19 mg., 0.18 millimoles) in glacial acetic acid (4.5 ml.) and water (0.5 ml.) was added to a solution of ester M (55 mg., 0.18 millimoles) in glacial acetic acid (5 ml.). The reaction solution was kept at room temperature for 48 hours and then methanol (10 ml.) was added to the green solution to reduce any excess chromium trioxide that might still have been present. The solution was then evaporated to dryness in a stream of nitrogen, under reduced pressure, to yield a dark green solid. An aqueous solution of the green solid was continuously extracted with ether for 3 days. The ether was evaporated at 30° under reduced pressure and the water present in the extract was azeotropically removed with benzene and 98% ethanol to yield a brown oil (53 mg.). The oil was washed with acetone. The acetone insoluble residue was a white powder (10 mg.) whose infrared spectrum (nujol) was identical to that of a sample of ester M. Removal of the acetone from the acetone wash at 30° under reduced pressure yielded a dark brown oil (38 mg.) whose infrared spectrum

(nujol) was very poorly resolved. All of the peaks in the spectrum were present in a spectrum of ester M.

The aqueous solution that had been continuously extracted with ether was basified with concentrated ammonium hydroxide and was then continuously extracted with ether for a further 4 days. The extract was evaporated to dryness to yield a brown oil. The oil crystallised from acetone as white microcrystals (12 mg.) of ester M (identified by m.p. and I.R. (nujol)).

The overall recovery of crude ester M was 91%.

B. At 100°.

An orange solution of chromium trioxide (2 g., 19 millimoles) in glacial acetic acid (90 ml.) and water (10 ml.) was added in small portions to a well stirred solution of ester M (780 mg., 2.5 millimoles) in glacial acetic acid (100 ml.) until the reaction solution remained an orange-green colour for 2 hours.

The reaction solution was evaporated to dryness at 60° by a stream of nitrogen under reduced pressure, to yield a dark green solid. Chloroform was added to the solid and the resulting mixture was refluxed with stirring for 14 hours. Filtration of the mixture, followed by evaporation of the filtrate to dryness, at 30° under reduced pressure yielded a green solid that was mainly inorganic in nature. The green solid and the chloroform insoluble material were therefore dissolved in absolute methanol and the resulting solution was saturated with hydrogen chloride. The solution was refluxed

for 17 hours using a condenser that was fitted with a calcium chloride guard tube, cooled, filtered and evaporated to dryness at 30° under reduced pressure to yield a green solid. An aqueous solution (p.H. 6) of the green solid was ether and methylene chloride extracted. The combined extracts were washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil (230 mg.). The aqueous acidic solution was basified with concentrated ammonium hydroxide and extracted with ether and methylene chloride. The combined extracts were washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil (160 mg.). The first and second brown oils were combined because their infrared spectra ($CHCl_3$) were identical.

A benzene solution of the combined brown oils was chromatographed on neutral 'Woelm' alumina (10 g.) Benzene elution yielded a white solid, the hydroxyketoester lactam M. Recrystallisation of the solid from ether yielded white microcrystals, which distilled at 160°, <u>in vacuo</u>, to yield a white, analytically pure distillate, m.p. 158°, I.R. (nujol) 3280 $cm.^{-1}$ (OH), 1726 $cm.^{-1}$ (-COO Me), 1696 $cm.^{-1}$ (ketone in 6 - membered carbocyclic ring) and 1673 $cm.^{-1}$ (δ lactam), (see I.R. -11), u.v. (95% EtoH), λ (inflection) 234 mμ. Analysed as $C_{16}H_{19}O_5N$. Calculated:- C, 62.95%; H, 6.27%; O, 26.20%; and N, 4.59%. Found:- C, 62.79%; H, 6.26%; O, 26.54%; and N, 4.65%. (See I.R. -12).

In a subsequent preparation the esterification proceedure was omitted and the lactam was isolated directly from the initial aqueous acidic solution by chloroform extraction. A further yield was obtained by basifying the acidic solution with ammonium hydroxide and extracting with chloroform. The lactam obtained was identical to the previously isolated lactam since it melted at the same temperature, exhibited an identical infrared spectrum (nujol) and a methanolic solution of it could not be esterified by treatment with ethereal diazomethane.

ANNOTININE BROMOHYDRIN HYDROBROMIDE, LXXV. A solution of annotinine (3.155 g.) in the minimum of aqueous hydrobromic acid (48%) was refluxed until cubic crystals precipitated out of solution. The mixture was cooled at 0° and then filtered to yield dark brown cubes. The cubes were well washed with acetone and large, glistening colourless cubes (3.900 g.) of annotinine bromohydrin hydrobromide were obtained. Recrystallisation of the cubes from methanol-acetone yielded colourless prisms, m.p. 278°, I.R. (nujol) 3430 cm.$^{-1}$ (OH), 2670 cm.$^{-1}$ ($-\overset{+}{N}-H$), 2590 cm.$^{-1}$ ($-\overset{+}{N}-H$) and 1796 cm.$^{-1}$ (γ lactone).

Slow evaporation of the hydrobromic acidic mother liquor yielded a solid, which after being washed with cold acetone gave colourless cubes (761 mg.) of annotinine bromohydrin hydrobromide, m.p. 274°.

The overall yield of annotinine bromohydrin hydrobromide was 4.661 g., 98.7%.

ANNOTININE BROMOHYDRIN, LXXVI. Chloroform was added to a mixture of annotinine bromohydrin hydrobromide (30 mg.) and concentrated ammonium hydroxide. The mixture was shaken and the chloroform removed. The chloroform solution was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield white prisms (24 mg., 98% yield) of annotinine bromohydrin m.p. 206°-207°, I.R. ($CHCl_3$) 3645 cm.$^{-1}$ (OH) and 1780 cm.$^{-1}$ (γ lactone, I.R. (nujol) 3450 cm.$^{-1}$ (OH) and 1760 cm.$^{-1}$ (γ lactone).

ATTEMPTED FORMATION OF ACETOXYANNOTININE BROMOHYDRIN, LXXVIIA.

A. Annotinine bromohydrin hydrobromide (100 mg.) was dissolved in boiling pyridine (5 ml.). The solution was cooled and acetic anhydride (5 ml.) was added to it. The reaction solution was stored in a flask that was fitted with a calcium chloride guard tube, at room temperature, for 24 hours.

The claret coloured reaction solution was evaporated to dryness at 30° *in vacuo* to yield a claret coloured oil (223 mg.). A chloroform solution of the oil was washed with dilute ammonium hydroxide and then water. It was dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil (93 mg.). The brown oil could not be solidified or crystallised.

Dilute hydrochloric acid (6 N, 0.2 ml.) was added to a solution of the brown oil in acetone. Evaporation of the solution to dryness at 30° under reduced pressure yielded

another brown oil. The oil could not be solidified or crystallised.

The oily salt was reconverted to the free base by shaking a chloroform solution of the salt with dilute ammonium hydroxide. The chloroform solution was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness at 30° under reduced pressure to yield a brown oil (89 mg.).

A solution of the free base in benzene was chromatographed on neutral "Woelm" alumina (grade I, 1.9 g.). Benzene elution yielded a pale yellow intractable oil (34 mg.), I.R. ($CHCl_3$) 1780, 1739 and 1252 cm.$^{-1}$. Ether-chloroform (1:1) elution yielded a brown intractable oil (32 mg.), I.R. ($CHCl_3$) 3300 - 3600, 1739, 1672 and 1252 cm.$^{-1}$ and chloroform-methanol(19:1) elution yielded an intractable brown oil (14 mg.), I.R. ($CHCl_3$), 3400, 1739, 1673 and 1254 cm.$^{-1}$.

ACTION OF ZINC AND GLACIAL ACETIC ACID ON ANNOTININE BROMOHYDRIN.

A. The method of Fieser and Ettorre (29) was used.
Activated zinc dust (800 mg., 12 millimoles) was added to a solution of annotinine bromohydrin (230 mg., 0.65 millimoles) in glacial acetic acid (35 ml.). The colourless solution was gently refluxed for 35 minutes, by which time a white crystalline precipitate was present.

The reaction mixture was filtered to yield white needles

(130 mg.) of zinc acetate, m.p. 238° (lit, 242°). The filtrate was diluted with water, basified with concentrated ammonium hydroxide and then chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness at 30° under reduced pressure to yield a white powder. The white powder was shown to be annotinine bromohydrin (150 mg., 65% recovery) by its m.p. and its infrared spectrum ($CHCl_3$).

B. The experiment 'A' was repeated, using a vast excess of zinc dust (10.2 g., 157 millimoles) and a small amount of annotinine bromohydrin (260 mg.). The solution of the 2 solids in glacial acetic acid was refluxed for 52 hours instead of 35 minutes.

The reaction mixture was worked up in the same manner as that used in 'A', to yield annotinine bromohydrin (197 mg., 76% recovery).

C. Experiment 'A' was repeated using 200 mg. of annotinine bromohydrin and a refluxing time of 12 hours instead of 35 minutes.

The reaction mixture was filtered and the filtrate evaporated to dryness at 60° in vacuo, to yield a white powder (410 mg.). The powder was added to n heptane and the resulting mixture was refluxed with stirring for 30 minutes. The mixture was filtered and the filtrate was evaporated to dryness at 30° under reduced pressure to yield a colourless oil (22 mg.). The infrared spectrum ($CHCl_3$) of

the oil was identical to that of the heptane insoluble material and so was added to the insoluble material again.

A chloroform solution of the combined material was shaken with dilute hydrochloric acid (3 N) and the acidic solution was then basified with concentrated ammonium hydroxide and chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil (190 mg.). The infrared spectrum ($CHCl_3$) of the oil was identical to that of the crude acetoxyannotinine bromohydrin obtained by the action of pyridine and acetic anhydride on annotinine bromohydrin hydrobromide.

ACTION OF ZINC IN ETHANOL ON ANNOTININE BROMOHYDRIN. The method used was that of Fieser and Etorre (29).

Annotinine bromohydrin (230 mg., 0.65 millimoles) was dissolved in a minimum of 98% ethanol and granulated zinc (3.4 g., 52 millimoles) was added to the resulting solution. The reaction mixture was refluxed and stirred for 9 hours, after which time a white precipitate was present in an otherwise clear solution.

The reaction mixture was filtered but the precipitate went through the paper. Therefore, all the solvent was evaporated at 60° under reduced pressure to yield a white solid. A mixture of chloroform and dilute ammonium hydroxide was added to the solid which immediately dissolved. The 2 solvents were shaken together and separated. The chloroform solution was washed with water, dried over anhydrous

sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a white solid (85 mg.) m.p. 154°. A solution of the white solid in benzene was chromatographed on neutral 'Woelm' alumina (grade I, 1 g.). Benzene elution yielded white needles (79 mg.) which were recrystallised from ether as white stars of Marion's (28) hydroxy lactone, m.p. 174° (lit. 174°), I.R. (nujol) 3150 cm.$^{-1}$ (OH) and 1772 cm.$^{-1}$ (γ lactone). The yield of hydroxylactone was 45%.

ACTION OF ZINC IN ACETIC ANHYDRIDE ON ANNOTININE BROMOHYDRIN.
Zinc dust (1 g. portions) was added every 2 hours to a solution of annotinine bromohydrin (320 mg.) in acetic anhydride (60 ml.) that was being stirred and refluxed. The first 3 g. added dissolved to give a light brown solution. A further 3 g. were added and the resulting mixture was then refluxed for 12 hours.

The excess zinc was filtered from the reaction mixture and washed with chloroform. The filtrate and the wash were combined and concentrated ammonium hydroxide was added to the combined solution until it was basic. Extraction of the basic mixture with chloroform, yielded a colourless extract, which was washed with water, dried over anhydrous sodium sulphate and then reduced to small volume. Straw coloured needles (820 mg.) of zinc acetate precipitated out of the chloroform solution and were filtered off and the filtrate shaken with dilute hydrochloric acid.

The chloroform solution was evaporated to dryness at 30° under reduced pressure to yield a brown oil (393 mg.). Two drops of concentrated hydrochloric acid were added to a solution of the brown oil in acetone. Evaporation of the acetone at 30° under reduced pressure yielded a black oil (413 mg.) whose infrared spectrum ($CHCl_3$) did not exhibit any peaks that could be characteristic of $-\overset{+}{N}-H$ stretching vibrations.

Concentrated ammonium hydroxide was added to the hydrochloric acid extract until it was basic. It was then chloroform extracted and the extract was washed with water and dried over anhydrous sodium sulphate. Evaporation of the chloroform at 30° under reduced pressure yielded a brown oil (38 mg.) which appeared to be a mixture of annotinine bromohydrin and O,N diacetylhydroxylactone. I.R. ($CHCl_3$) 3570, 3430, 1775, 1730 and 1675 cm^{-1}.

O,N - DIACETYLHYDROXYLACTONE. The method of Anet and Marion (39) was used.

A solution of the hydroxylactone (30 mg.) in acetic anhydride (2 ml.) was heated for 3 hours at 100°.

The light brown reaction solution was diluted with water (5 ml.) and stirred for 2 hours at room temperature. The aqueous solution was ether extracted and the extract was washed with aqueous sodium bicarbonate and then with water. It was dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield O,N,-diacetylhydroxylactone as a pale yellow solid (24 mg., 60% yield),

m.p. 119°-120° (lit. 124°-126°), I.R. ($CHCl_3$) 1768 $cm.^{-1}$ (δ lactone), 1736 $cm.^{-1}$ (-O -Ac), 1662 $cm.^{-1}$ (-N -Ac) and 1250 $cm.^{-1}$ (-O -Ac). (See I.R. -13.).

ACTION OF ZINC IN ETHANOL ON ACETOXYANNOTININE BROMOHYDRIN. Zinc dust (4 g., 62 millimoles) was added to a solution of crude, oily acetoxyannotinine hydrobromide (372 mg., 0.93 millimoles) in 98% ethanol. The mixture was refluxed and stirred for 2 days.

The reaction mixture was filtered and the clear yellow filtrate was evaporated to dryness at 60° under reduced pressure to yield starting material as a light brown, oily solid (352 mg., 95% recovery). The oily solid was identified by its infrared spectrum ($CHCl_3$).

ATTEMPTED METHYLATION OF ANNOTININE BROMOHYDRIN. The method of Roberts and Johnson (40) was used.

A solution of annotinine bromohydrin (70 mg., 0.4 millimoles) in chloroform was added to a solution of fluoroboric acid (206 mg., 5.96 millimoles) in ether. The solution was stirred and cooled in an acetone-dry ice bath, while diazomethane was slowly added over a period of 5 minutes. A white precipitate was obtained. The reaction mixture was filtered and washed with a little chloroform. The filtrate was refiltered. The solid from the first filtration was a white powder (45 mg.) m.p. 176°, I.R. (nujol) 3560, 3150, 1782 and 1767 $cm.^{-1}$.

The solid obtained from the second filtration was a

pale yellow powder (41 mg.) I.R. ($CHCl_3$) 2640, 3510, 1777 and 1730 cm.$^{-1}$. The powder was washed with ether and the residue was crystallised from hot ethyl acetate as pale yellow prisms (34 mg., 48% recovery) of annotinine bromohydrin, m.p. 207°. The infrared spectrum (nujol) was identical to that of annotinine bromohydrin.

DEHYDRODESOXIDOANNOTININE, LXXVIII. The method of L. Marion (28) was used.

Annotinine bromohydrin (7.561 g., 21.3 millimoles) as a suspension in water (10 ml.) and concentrated hydrochloric acid (26 ml.) was introduced into a 3-necked flask that was fitted with a 'Jone's Reductor', a nitrogen inlet and a condenser. The suspension was stirred and purified (oxygen free) nitrogen was passed through the suspension for 15 minutes. Suction was applied at the top of the condenser and a solution of chromic chloride hexahydrate (49.46 g., 179.5 millimoles of $CrCl_2$) in water (280 ml.) and concentrated hydrochloric acid (4 ml.) was slowly passed through the Jones Reductor and into the suspension. The blue green solution was refluxed for 3 hours.

The green reaction solution was basified with concentrated ammonium hydroxide and was then ether (20 l.) extracted. The extract was washed with water, dried over anhydrous magnesium sulphate and reduced to a small volume. White prisms (1.713 g., 36% yield) of the hydroxylactone were obtained m.p. 172° (lit 174°).

The ethereal solution, from which the hydroxylactone

had been obtained, was evaporated to dryness to yield a light brown oil. A solution of the oil in benzene was chromatographed on neutral "Woelm" alumina (grade I, 8 g.). Elution with benzene (1200 ml.) yielded dehydrodesoxidoannotinine (1.331 g., 30% yield) as a white solid. Dehydrodesoxidoannotinine was crystallised from n heptane as colourless prisms, m.p. 128°-129° (lit 128°-130°), I.R. ($CHCl_3$) 1780 $cm.^{-1}$ (γ lactone). (See I.R. -14).

DESOXIDOANNOTININE, LXXX. Dehydrodesoxidoannotinine (420 mg., 1.518 millimoles) was added to a well stirred suspension of pre-reduced platinum oxide (390 mg.) in 98% ethanol (15 ml.) at room temperature, in an atmosphere of hydrogen. After 70 minutes absorption of hydrogen stopped. The uptake was 48 ml., 1.2 millimoles.

The reaction mixture was filtered and the colourless filtrate evaporated to dryness at 60° under reduced pressure to yield a white, semi-solid 428 mg. The semi-solid crystallised from Skelly solve B as colourless, small needles of desoxidoannotinine (393 mg., 93% yield) m.p. 108.5°-109.5° (lit. 110°), I.R. (nujol) 1772 $cm.^{-1}$ (δ lactone) (lit. 1768 $cm.^{-1}$). (See I.R. - 15).

DESOXIDOANNOTINIC ACID, LXXXI. A solution of barium hydroxide (2.5 g.) in water (75 ml.) was added to a solution of desoxidoannotinine (340 mg.) in 98% ethanol (75 ml.). The colourless solution was stirred and refluxed for 4 hours using a condenser that was fitted with an ascarite guard tube, cooled and saturated with carbon dioxide. The precipitated barium carbonate

was filtered off and washed with ethanol. Further carbon dioxide was added to the filtrate, but it remained perfectly clear. The wash and the filtrate were mixed and evaporated to dryness at 60° under reduced pressure to yield a light brown semi-solid (480 mg.) The semi-solid could not be crystallised and its infrared spectrum (nujol) was very poorly resolved but did exhibit peaks at 3350, 3330, 3670, 2570, 1752 and 1700 $cm.^{-1}$, which indicated that the semi-solid was impure desoxidoannotininic acid. The compound was crystallised as its crystalline methyl ester.

METHYL DESOXIDOANNOTINATE, LXXXII. An ethereal solution of diazomethane was added to a methanolic solution of desoxidoannotinic acid (480 mg.) until the solution was a permanent yellow colour.

The reaction solution was filtered and the filtrate evaporated to dryness at 30° under reduced pressure to yield a brown semi-solid (490 mg.), I.R. ($CHCl_3$) 3650, 3460, 1770 and 1725 $cm.^{-1}$.

A benzene solution of the semi-solid was chromatographed on neutral "Woelm" alumina (grade I, 10 g.). Elution with ether (150 ml.) yielded a colourless viscous oil (153 mg., 30% yield) which was crystallised 6 times from ethyl acetate-ether to give analytically pure, colourless needles of methyl desoxidoannotinate, m.p. 166°, I.R. (nujol) 3170 $cm.^{-1}$ (OH) and 1738 $cm.^{-1}$ (-COO Me) I.R. ($CHCl_3$) 3660 $cm.^{-1}$ (OH), 3510 $cm.^{-1}$ (OH) and 1730 $cm.^{-1}$ (-COO Me). (See I.R. -16). Analysed as $C_{17}H_{27}O_3N$.

CALCULATED:- C, 69.59%; H, 9.21%; O, 16.37%; N, 4.78% and -O -Me, 10.58%. Found:- C, 68.98%; H, 9.14%; O, 16.48%; N, 4.81% and -O -Me, 10.46%.

ATTEMPTED PREPARATION OF METHYL <u>EPI</u>DESOXIDOANNOTINATE, LXXXIII. Desoxidoannotinine (200 mg., 0.77 millimoles) was dissolved in a solution of potassium (200 mg., 5.13 millimoles) in absolute methanol (10 ml.). The solution was stored at room temperature in a flask that was fitted with a calcium chloride guard tube for 4 days.

The reaction solution was evaporated to dryness at 30° under reduced pressure and finally *in vacuo*, to yield a yellow solid. The yellow solid was dissolved in ice-cold water and the resulting solution was chloroform extracted. Water was used to wash the extract, which was then dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil. The infrared spectrum ($CHCl_3$) of the oil was identical to the spectrum of the starting material except for weak absorption at 1725 $cm.^{-1}$.

METHYL <u>EPI</u>DESOXIDOANNOTINATE, LXXXIII. Desoxidoannotinine (200 mg., 0.77 millimoles) was dissolved in a solution of potassium (200 mg., 5.13 millimoles) in absolute methanol (20 ml.). The solution was refluxed for 26 hours using a condenser that was fitted with a calcium chloride guard tube.

Evaporation of the methanol from the reaction solution,

initially at 30° under reduced pressure and finally *in vacuo*, yielded a brown oil (197 mg.) I.R. ($CHCl_3$) 3690, 3510, 1779 and 1726 $cm.^{-1}$.

A solution of the oil in benzene was chromatographed on neutral "Woelm" alumina (2.5 g.). Elution with ether-benzene (97:3) yielded a yellow oil (110 mg.), I.R. ($CHCl_3$) 3490, 3450, 1776 and 1725 $cm.^{-1}$. The peak at 1776 $cm.^{-1}$ was very weak in intensity.

A solution of the yellow oil in ether-benzene (9:1) was chromatographed on neutral "Woelm" alumina (grade I, 2.3 g.). Elution with ether-benzene (97:3) yielded a cream coloured oil (37 mg.) that still exhibited weak absorption at 1776 $cm.^{-1}$ in its infrared spectrum ($CHCl_3$).

A solution of the cream coloured oil in ether-benzene (5%) was chromatographed on neutral "Woelm" alumina (grade I, 1 g.). Elution with ether-benzene (193:7) yielded a white solid (23 mg.). The white solid was crystallised 3 times from ether-pentane and sublimed twice at 155°, to yield analytically pure white cubes of methyl *epi*desoxidoannotinine, m.p. 198.5°, I.R. ($CHCl_3$) 3650 $cm.^{-1}$ (OH), 3640 $cm.^{-1}$ (OH) and 1724 $cm.^{-1}$ (-COO Me). (See I.R. -17). Analysed as $C_{17}H_{27}O_3N$ Calculated:- C, 69.59%; H, 9.21%; O, 16.37%; N, 4.78% and O-Me, 10.58%. Found:- C, 69.11%; H, 9.33%; O, 16.80%; N, 4.88% and O-Me 10.55%.

EPIMERISATION OF METHYL DESOXIDOANNOTINATE. Methyl desoxidoannotinate (200 mg., 0.69 millimoles) was dissolved in a

solution of potassium (200 mg., 5.28 millimoles) in absolute methanol (10 ml.). The solution was stored at room temperature for 6 days.

The reaction solution was evaporated to dryness at 30° under reduced pressure and finally *in vacuo*, to yield a yellow oil. An aqueous solution of the yellow oil was chloroform extracted. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure, to yield a yellow semi-solid. A benzene solution of the yellow semi-solid was chromatographed on neutral "Woelm" alumina. Elution with ether-benzene (97:3) yielded a brown oil, whose infrared spectrum ($CHCl_3$) was identical to that of methyl *epi*desoxidoannotinate.

USE OF FORCING CONDITIONS TO OPEN LACTONE RING OF DESOXIDOANNOTININE. Desoxidoannotinine (310 mg., 1.18 millimoles) was dissolved in a solution of sodium methoxide (3g., 37 millimoles) in absolute methanol (60 ml.) and the solution was refluxed for 45 hours, using a condenser that was fitted with a calcium chloride guard tube.

The reaction solution was evaporated to dryness at 30° under reduced pressure and finally *in vacuo* to yield a brown solid. The brown solid was dissolved in ice-cold water (20 ml.) and was then chloroform extracted. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at 30° under reduced pressure to yield a brown oil (109 mg.). The infrared spectrum

($CHCl_3$) of the brown oil was identical to that of methyl _epi_-desoxidoannotinate and did not exhibit a peak at 1780 cm.$^{-1}$.

ATTEMPTED CONVERSION OF THE HYDROXYLACTONE TO ANNOTININE BROMOHYDRIN. Liquid bromine was added dropwise to a solution of the hydroxy lactone (650 mg.) in chloroform until the solution was a permanent orange colour. The solution was evaporated to dryness at 30° under reduced pressure to yield an orange oil. A solution of the oil in 98% ethanol was then refluxed for 3 hours and was then evaporated to dryness at 60° under reduced pressure to yield an orange oil. The oil was dissolved in ether-chloroform (1:1) and the solution evaporated to dryness at 30° under reduced pressure to yield a pale yellow, oily solid (673 mg.,) I.R. (nujol) 3350, 1770 and 1588 cm.$^{-1}$. The spectrum was not identical to either annotinine bromohydrin or the hydroxylactone.

ATTEMPTED PREPARATION OF DIBROMOHYDROXYLACTONE. Liquid bromine was added dropwise to a solution of the hydroxylactone (24 mg.) in chloroform until the solution was a permanent orange colour.

The reaction solution was evaporated to dryness at 30° under reduced pressure to yield a red oil. Ether was added to the oil and was then removed at 30° under reduced pressure. The residue was a pale yellow solid (42 mg.) m.p. 101° - 107°, I.R. ($CHCl_3$) 3320, 1773 cm.$^{-1}$. The spectrum was not identical to either annotinine bromohydrin or the hydroxylactone.

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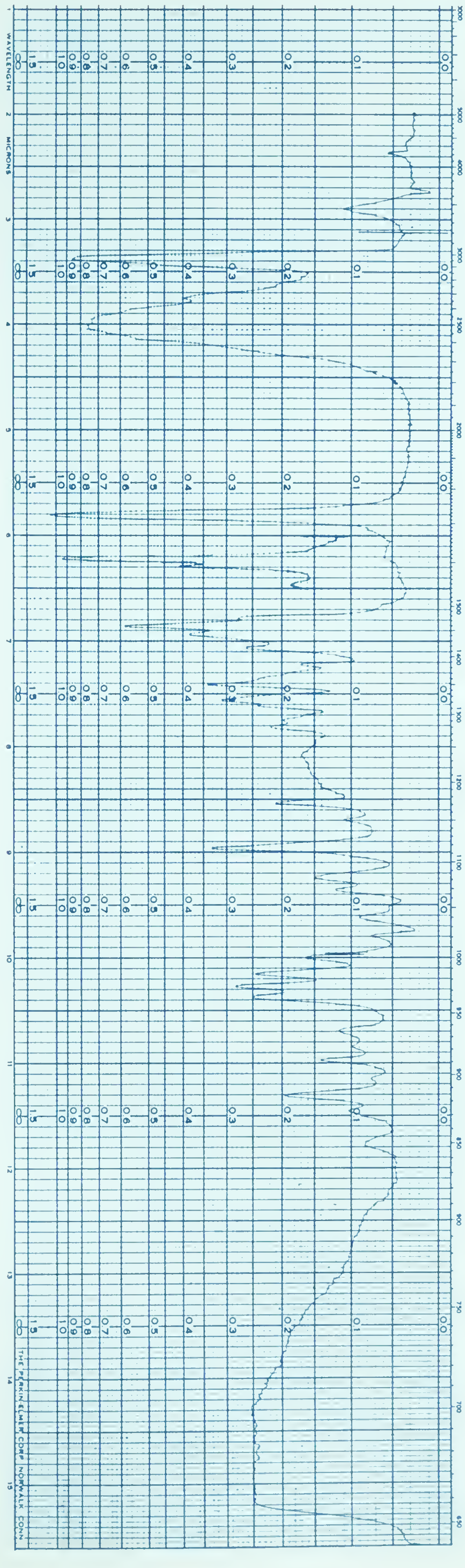
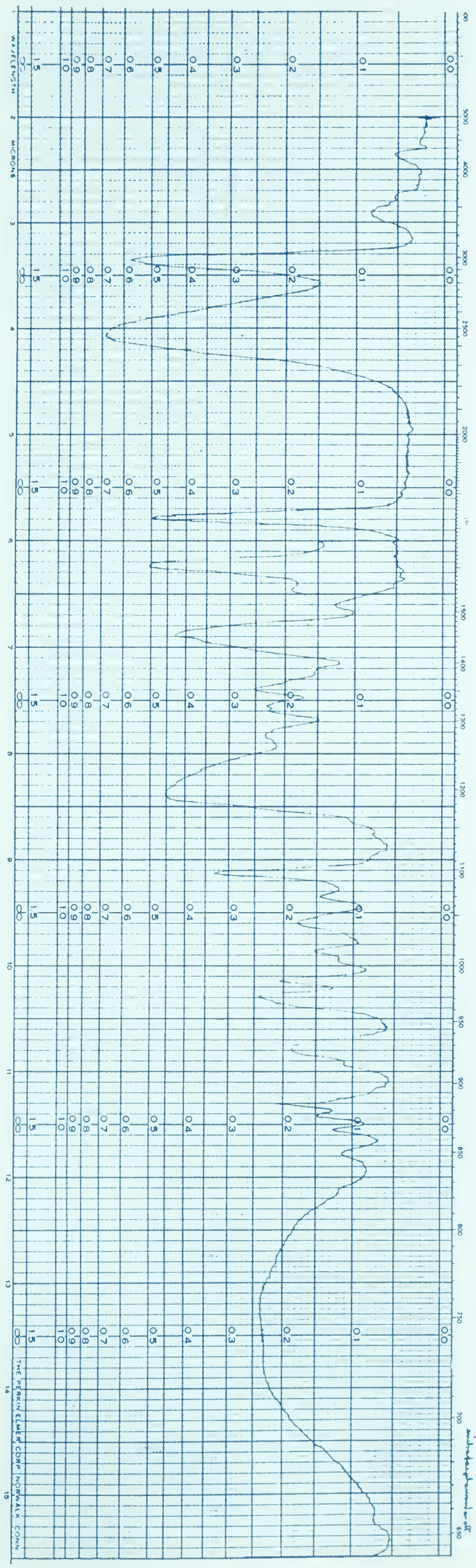
INFRARED SPECTRA.


WAVELENGTH MICRONS
THE PERKIN-ELMER CORP NORWALK CONN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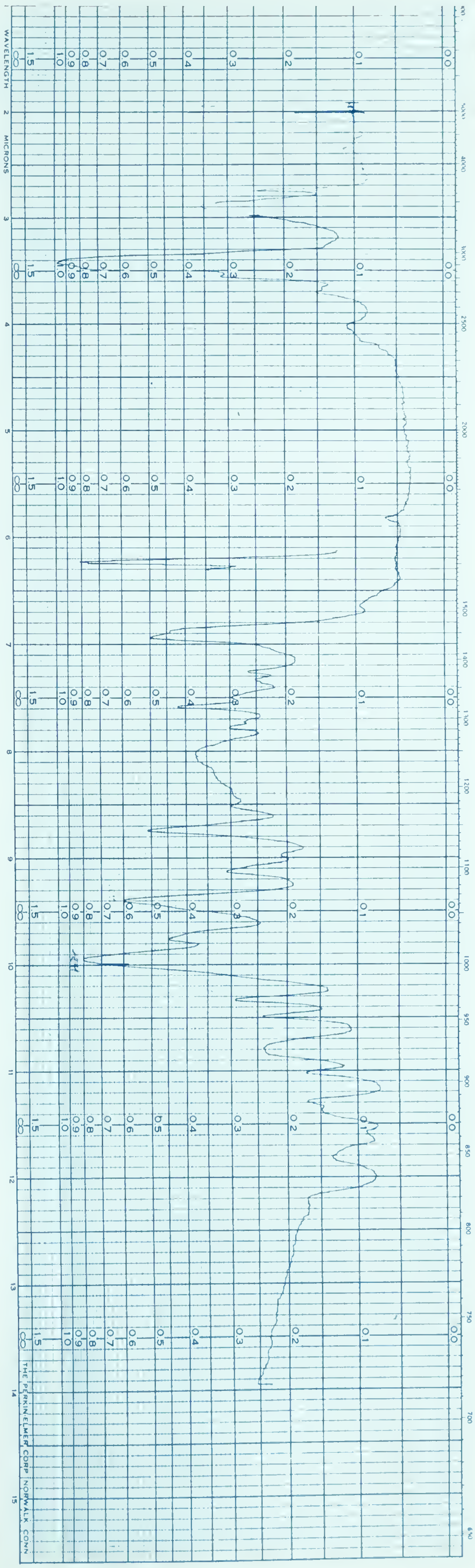
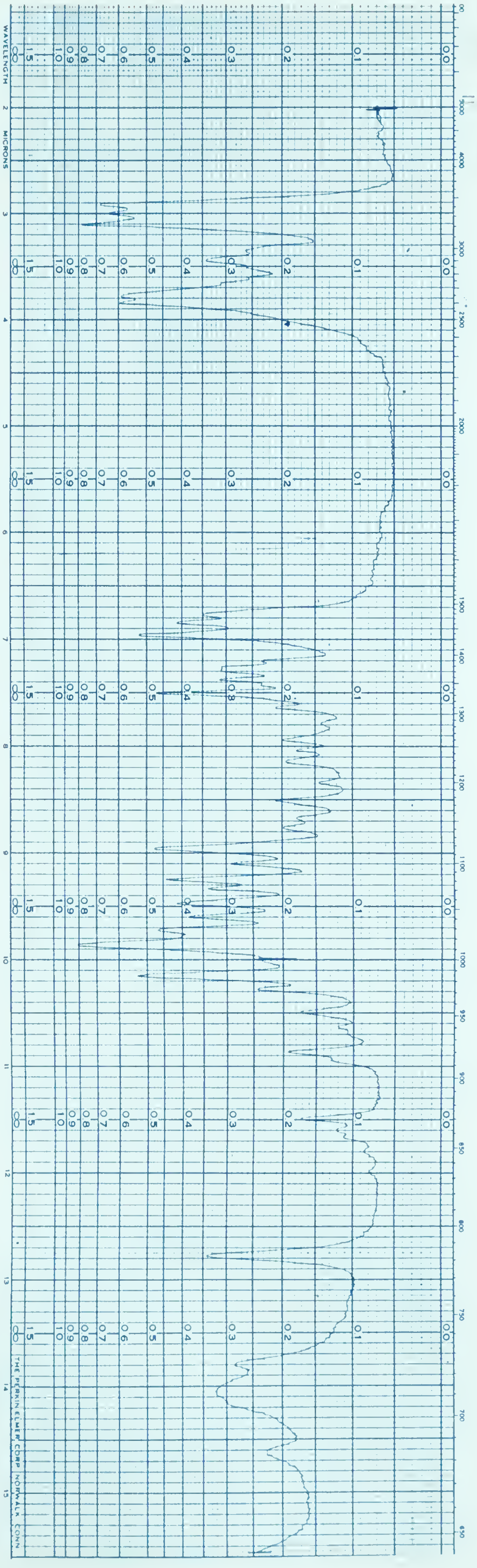

WAVELENGTH MICRONS
THE PERKIN-ELMER CORP NORWALK CONN

I.R. 4 (nujol)

[illegible]urobromide of the diol =

[illegible]ide.

[illegible](see 2[illegible]).

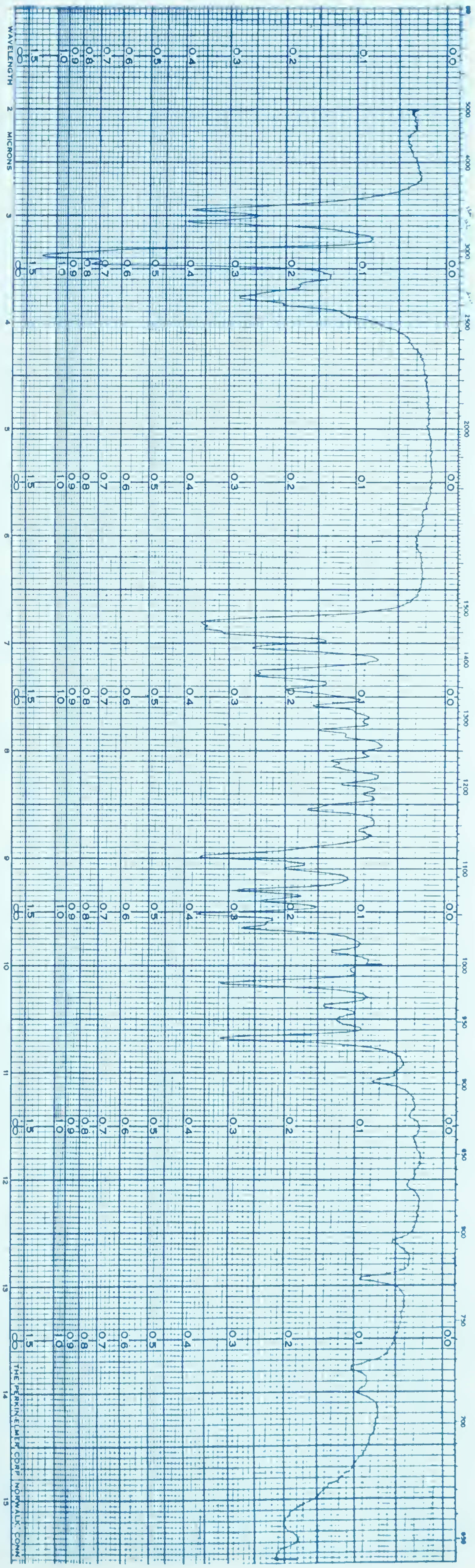


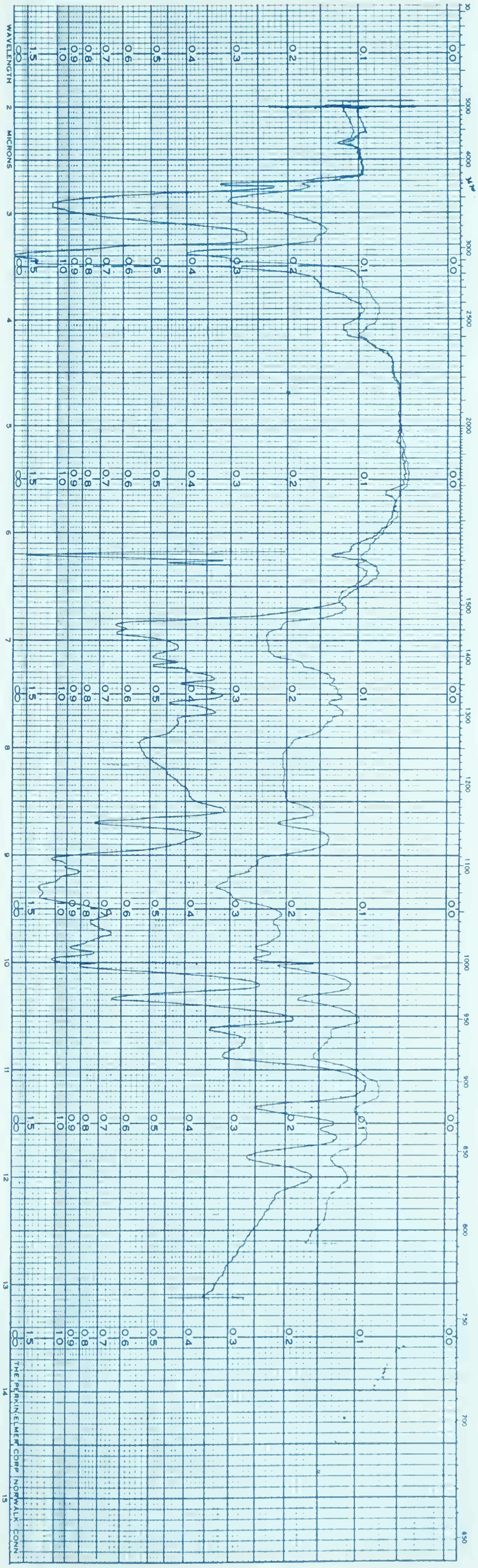
I.R. 5 (nujol)

Hydrochloride of the diste-

codeine.

[illegible] ([illegible]).

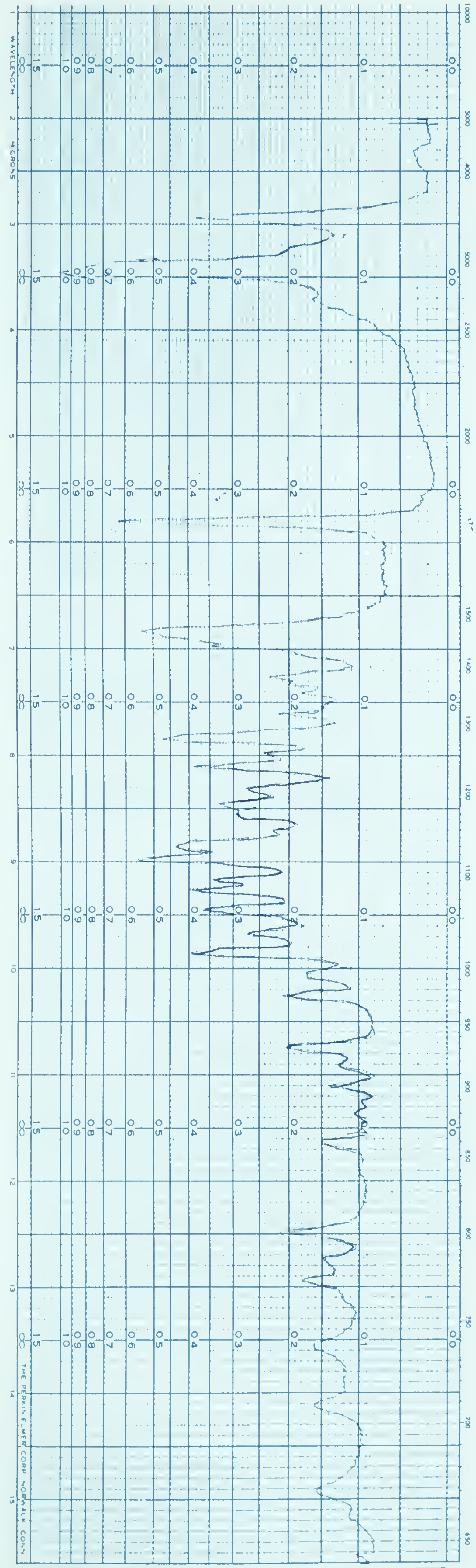



WAVELENGTH
MICRONS
THE PERKIN-ELMER CORP NORWALK CONN

[illegible] 7 (nujol)

[illegible]

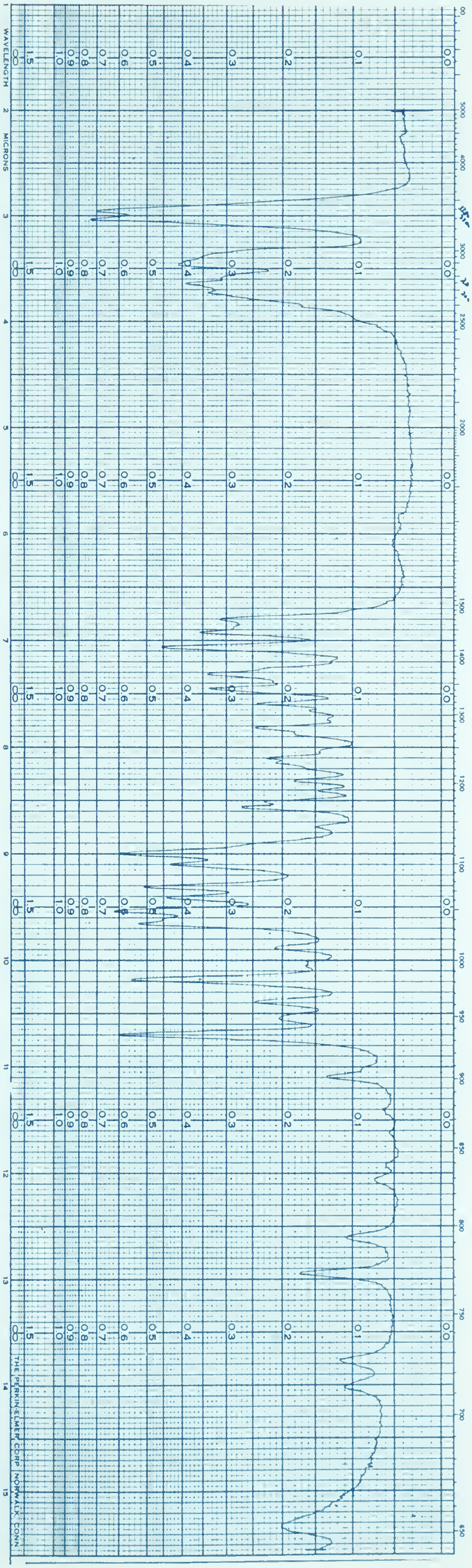
[illegible]

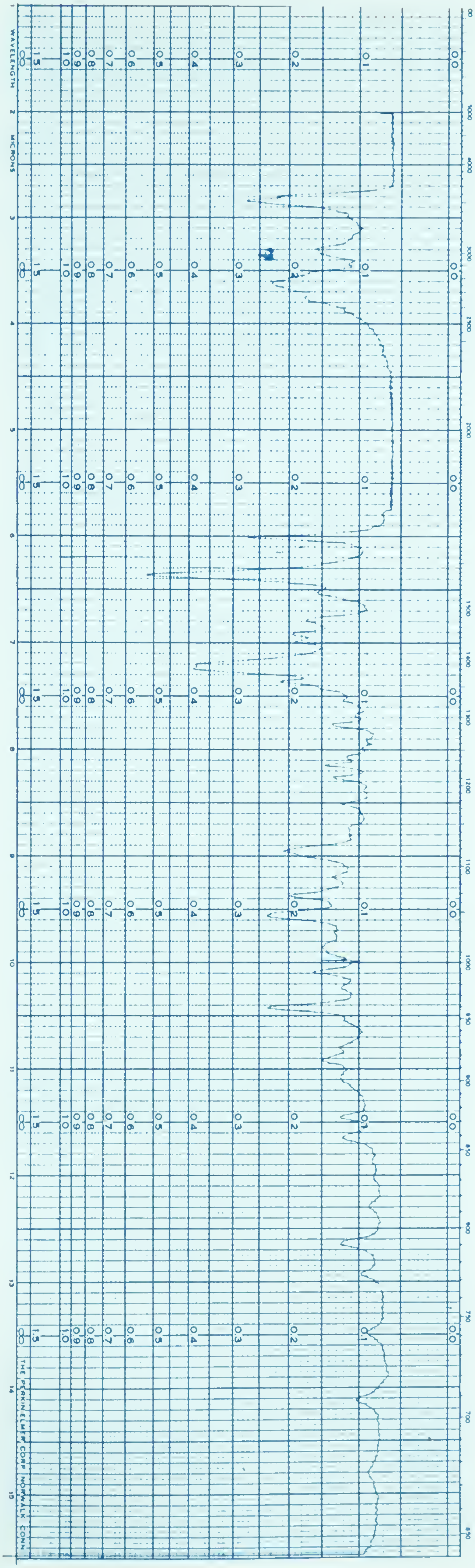


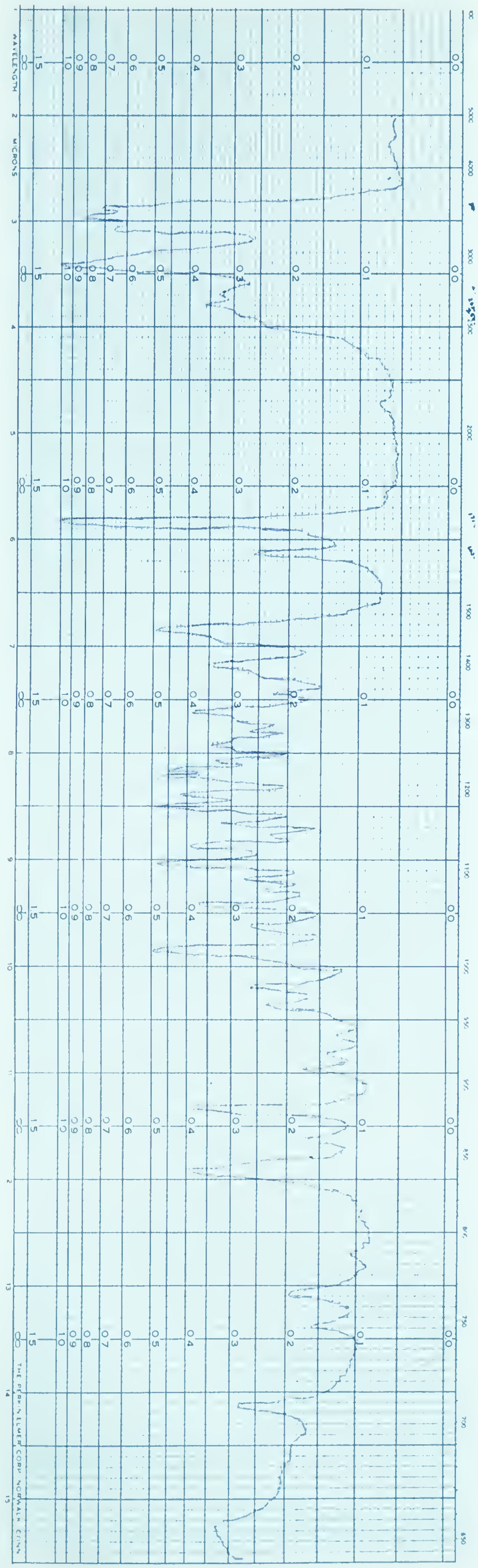
I. R. 3 (nujol)

Nujol m.

L.




WAVELENGTH MICRONS
THE PERKIN-ELMER CORP NORWALK CONN



I. [illegible]-10. (nujol)

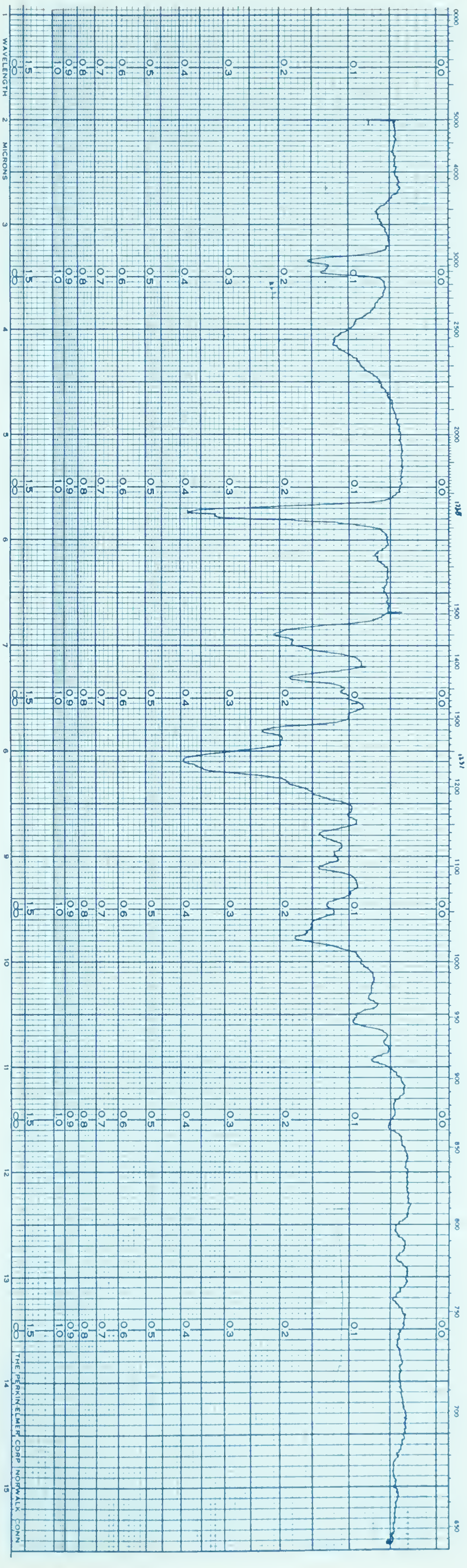
[illegible]cid [illegible]. Hydrochloride.

[illegible]LII.

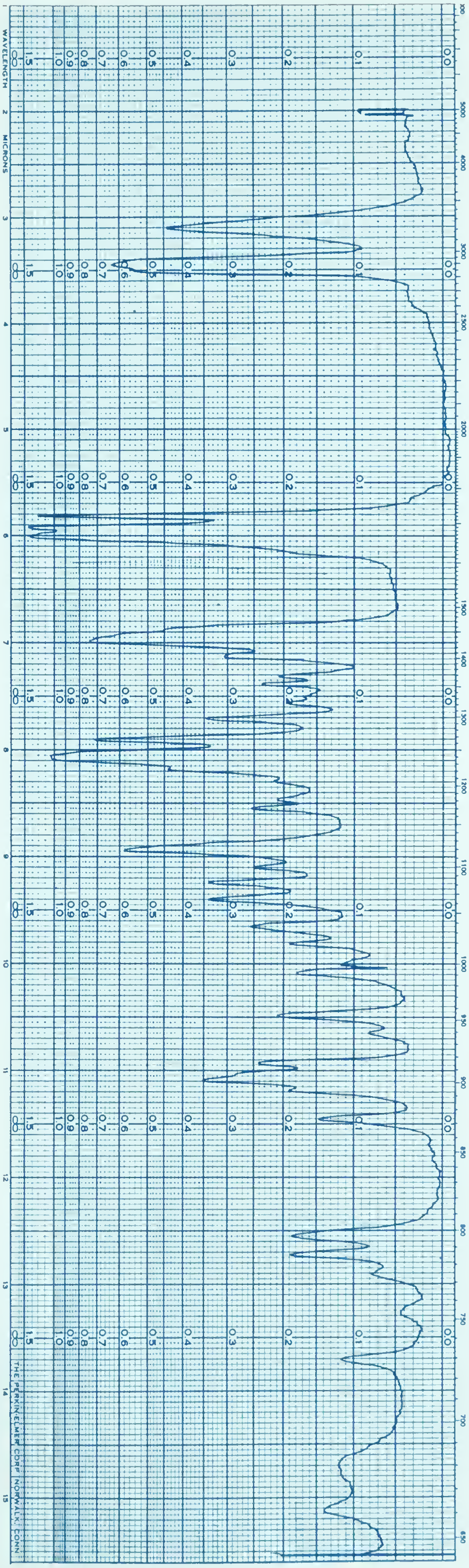
[illegible].II(nujol)

Ester [illegible]. Diacetate.

L[illegible].

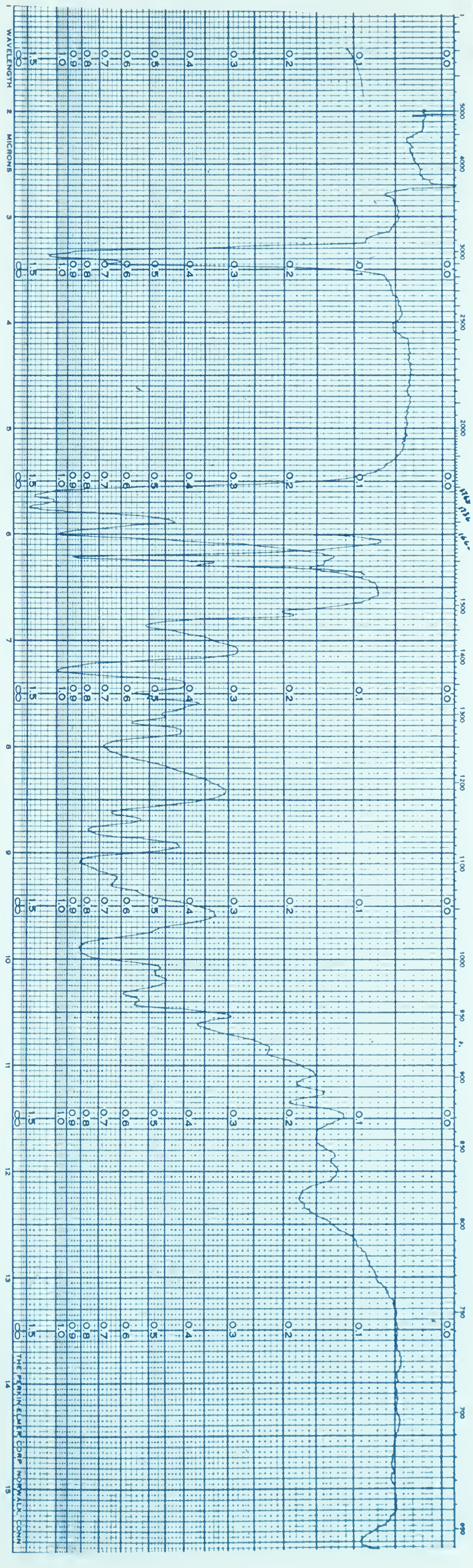


I.R. 12 (nujol)



I.R.-13 ($CHCl_3$)

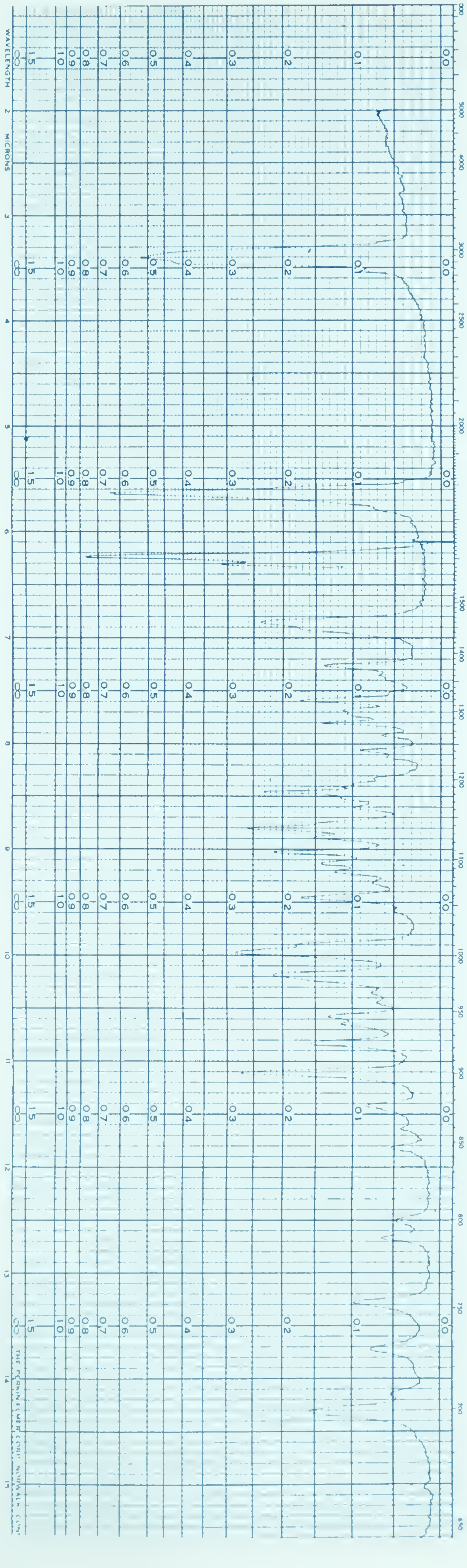
O.N.Di...-hydroxyl...

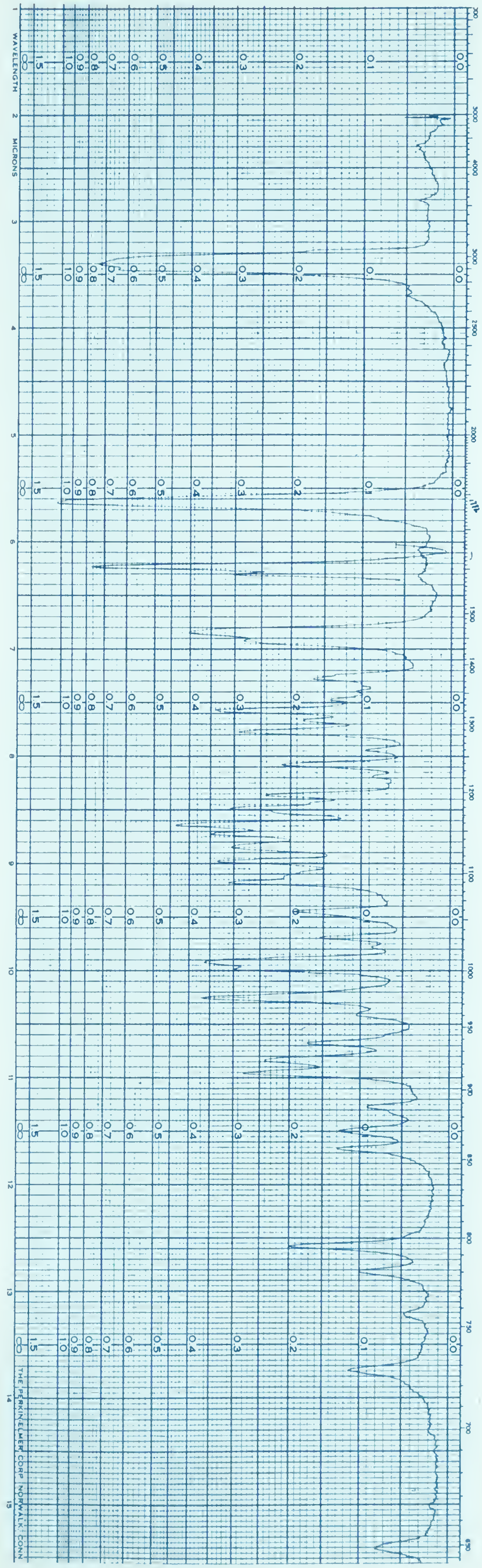


[illegible] (nujol)

[illegible]

LXVIII.



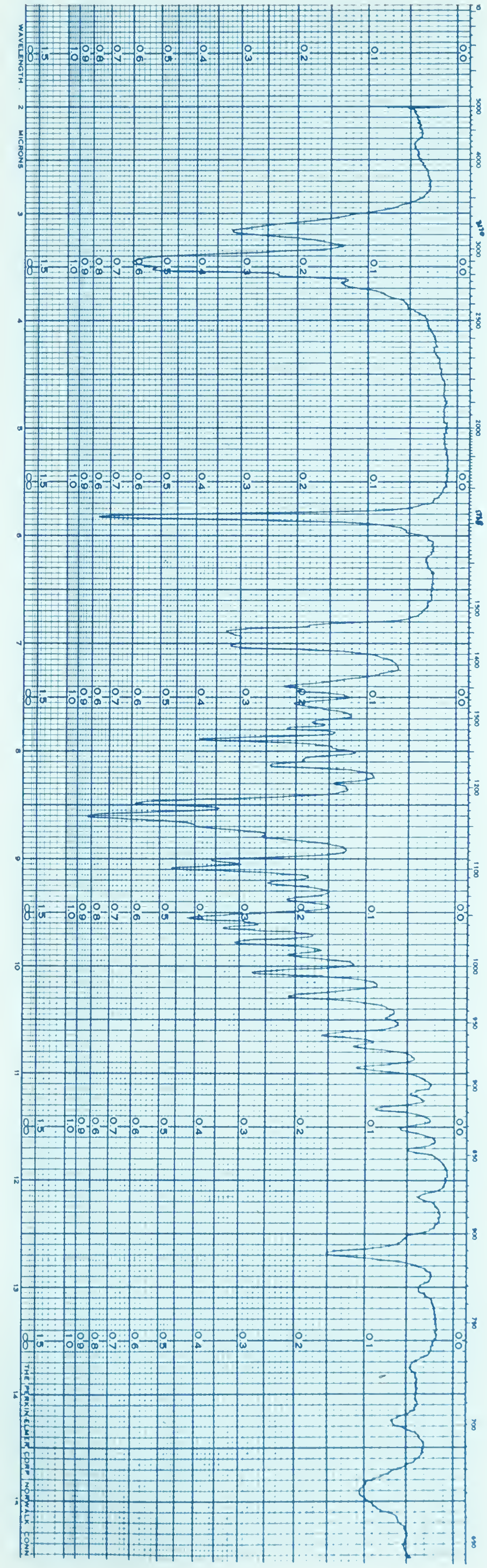


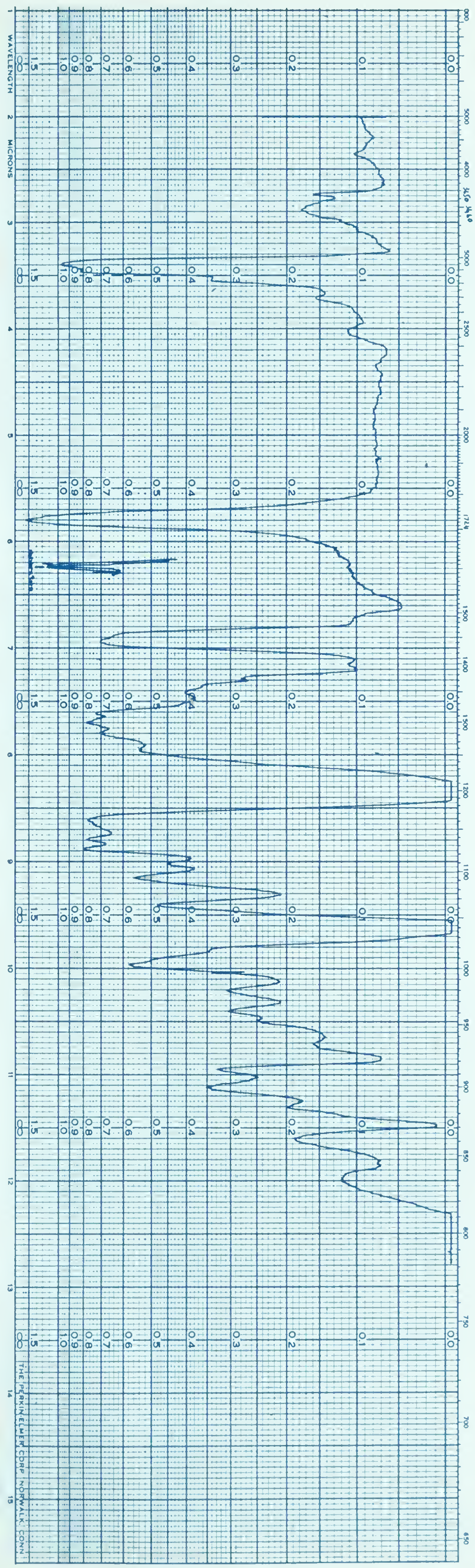
WAVELENGTH
MICRONS
THE PERKIN-ELMER CORP NORWALK CONN

I.R. 36 (nujol)

Methyl [illegible]

[illegible] III.





I. . 17 (CCl_4)

Methyl [illegible]
acid.

Band III.